



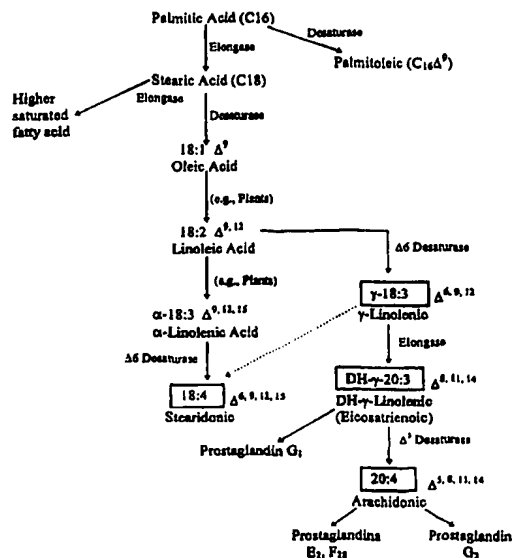
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|--|--|--|---|
| (51) International Patent Classification ⁶ : C12N 15/53, 15/82, 5/10, C12P 7/64, C11B 1/00, A61K 31/20, A23L 1/30, A23K 1/00 | | A1 | (11) International Publication Number: WO 98/46764 |
| | | | (43) International Publication Date: 22 October 1998 (22.10.98) |
| (21) International Application Number: PCT/US98/07421 | | ABBOTT LABORATORIES [US/US]; 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US). | |
| (22) International Filing Date: 10 April 1998 (10.04.98) | | (72) Inventors; and | |
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| 08/833,610 11 April 1997 (11.04.97) US 08/834,033 11 April 1997 (11.04.97) US 08/834,655 11 April 1997 (11.04.97) US 08/956,985 24 October 1997 (24.10.97) US | | (74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111-4262 (US). | |
| (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). | |
| US 08/834,655 (CIP) Filed on 11 April 1997 (11.04.97) US 08/833,610 (CIP) Filed on 11 April 1997 (11.04.97) US 08/834,033 (CIP) Filed on 11 April 1997 (11.04.97) US 08/956,985 (CIP) Filed on 24 October 1997 (24.10.97) | | Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. | |
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(54) Title: METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

(57) Abstract

The present invention relates to compositions and methods for preparing polyunsaturated long chain fatty acids in plants, plant parts and plant cells, such as leaves, roots, fruits and seeds. Nucleic acid sequences and constructs encoding fatty acid desaturases, including $\Delta 5$ -desaturases, $\Delta 6$ -desaturases and $\Delta 12$ -desaturases, are used to generate transgenic plants, plant parts and cells which contain and express one or more transgenes encoding one or more desaturases. Expression of the desaturases with different substrate specificities in the plant system permit the large scale production of polyunsaturated long chain fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, α -linolenic acid, gamma-linolenic acid, arachidonic acid and the like for modification of the fatty acid profile of plants, plant parts and tissues. Manipulation of the fatty acid profiles allows for the production of commercial quantities of novel plant oils and products.



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METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of USSN 08/834,655, filed
5 April 11, 1997, and a continuation in part of USSN 08/833,610, filed April 11,
1997, USSN 08/834,033 filed April 11, 1997 and USSN 08/956,985 filed
October 24, 1997 which disclosures are incorporated herein by reference.

INTRODUCTION

Field of the Invention

10 This invention relates to modulating levels of enzymes and/or enzyme
components capable of altering the production of long chain polyunsaturated
fatty acids (PUFAS) in a host plant. The invention is exemplified by the
production of PUFAS in plants.

Background

15 Two main families of polyunsaturated fatty acids (PUFAs) are the $\omega 3$
fatty acids, exemplified by arachidonic acid, and the $\omega 6$ fatty acids, exemplified
by eicosapentaenoic acid. PUFAs are important components of the plasma
membrane of the cell, where they may be found in such forms as phospholipids.
PUFAs also serve as precursors to other molecules of importance in human
20 beings and animals, including the prostacyclins, leukotrienes and
prostaglandins. PUFAs are necessary for proper development, particularly in
the developing infant brain, and for tissue formation and repair.

Four major long chain PUFAs of importance include docosahexaenoic
acid (DHA) and eicosapentaenoic acid (EPA), which are primarily found in
25 different types of fish oil, gamma-linolenic acid (GLA), which is found in the
seeds of a number of plants, including evening primrose (*Oenothera biennis*),
borage (*Borago officinalis*) and black currants (*Ribes nigrum*), and stearidonic
acid (SDA), which is found in marine oils and plant seeds. Both GLA and
another important long chain PUFA, arachidonic acid (ARA), are found in

filamentous fungi. ARA can be purified from animal tissues including liver and adrenal gland.

For DHA, a number of sources exist for commercial production including a variety of marine organisms, oils obtained from cold water marine fish, and egg yolk fractions. For ARA, microorganisms including the genera *Mortierella*, *Entomophthora*, *Phytium* and *Porphyridium* can be used for commercial production. Commercial sources of SDA include the genera *Trichodesma* and *Echium*. Commercial sources of GLA include evening primrose, black currants and borage. However, there are several disadvantages associated with commercial production of PUFAs from natural sources. Natural sources of PUFAs, such as animals and plants, tend to have highly heterogeneous oil compositions. The oils obtained from these sources therefore can require extensive purification to separate out one or more desired PUFAs or to produce an oil which is enriched in one or more PUFA. Natural sources also are subject to uncontrollable fluctuations in availability. Fish stocks may undergo natural variation or may be depleted by overfishing. Fish oils have unpleasant tastes and odors, which may be impossible to economically separate from the desired product, and can render such products unacceptable as food supplements. Animal oils, and particularly fish oils, can accumulate environmental pollutants. Weather and disease can cause fluctuation in yields from both fish and plant sources. Cropland available for production of alternate oil-producing crops is subject to competition from the steady expansion of human populations and the associated increased need for food production on the remaining arable land. Crops which do produce PUFAs, such as borage, have not been adapted to commercial growth and may not perform well in monoculture. Growth of such crops is thus not economically competitive where more profitable and better established crops can be grown. Large scale fermentation of organisms such as *Mortierella* is also expensive. Natural animal tissues contain low amounts of ARA and are difficult to process. Microorganisms such as *Porphyridium* and *Mortierella* are difficult to cultivate on a commercial scale.

Dietary supplements and pharmaceutical formulations containing PUFAs can retain the disadvantages of the PUFA source. Supplements such as fish oil capsules can contain low levels of the particular desired component and thus require large dosages. High dosages result in ingestion of high levels of
5 undesired components, including contaminants. Care must be taken in providing fatty acid supplements, as overaddition may result in suppression of endogenous biosynthetic pathways and lead to competition with other necessary fatty acids in various lipid fractions *in vivo*, leading to undesirable results. For example, Eskimos having a diet high in ω 3 fatty acids have an increased
10 tendency to bleed (U.S. Pat. No. 4,874,603). Unpleasant tastes and odors of the supplements can make such regimens undesirable, and may inhibit compliance by the patient.

A number of enzymes are involved in PUFA biosynthesis. Linoleic acid (LA, 18:2 Δ 9, 12) is produced from oleic acid (18:1 Δ 9) by a Δ 12-desaturase.
15 GLA (18:3 Δ 6, 9, 12) is produced from linoleic acid (LA, 18:2 Δ 9, 12) by a Δ 6-desaturase. ARA (20:4 Δ 5, 8, 11, 14) production from DGLA (20:3 Δ 8, 11, 14) is catalyzed by a Δ 5-desaturase. However, animals cannot desaturate beyond the Δ 9 position and therefore cannot convert oleic acid (18:1 Δ 9) into linoleic acid (18:2 Δ 9, 12). Likewise, α -linolenic acid (ALA, 18:3 Δ 9, 12, 15) cannot
20 be synthesized by mammals. Other eukaryotes, including fungi and plants, have enzymes which desaturate at positions Δ 21 and Δ 15. The major poly-unsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid (18:2 Δ 9, 12) or α -linolenic acid (18:3 Δ 9, 12, 15).

25 Poly-unsaturated fatty acids are considered to be useful for nutritional, pharmaceutical, industrial, and other purposes. An expansive supply of poly-unsaturated fatty acids from natural sources and from chemical synthesis are not sufficient for commercial needs. Therefore it is of interest to obtain genetic material involved in PUFA biosynthesis from species that naturally produce
30 these fatty acids and to express the isolated material alone or in combination in

a heterologous system which can be manipulated to allow production of commercial quantities of PUFAS.

The present invention is further directed to formulas, dietary supplements or dietary supplements in the form of a liquid or a solid containing
5 the long chain fatty acids of the invention. These formulas and supplements may be administered to a human or an animal.

The formulas and supplements of the invention may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed
10 whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

The formulas of the present invention may further include at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of
15 calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

The present invention is further directed to a method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient a dietary substitute of the
20 invention in an amount sufficient to effect treatment of the patient.

The present invention is further directed to cosmetic and pharmaceutical compositions of the material of the invention.

The present invention is further directed to transgenic oils in pharmaceutically acceptable carriers. The present invention is further directed
25 to nutritional supplements, cosmetic agents and infant formulae containing transgenic oils.

The present invention is further directed to a method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of: growing a microbe having cells which contain a transgene which encodes a

transgene expression product which desaturates a fatty acid molecule at carbon 5,5 or 12 from the carboxyl end of said fatty acid molecule, wherein the transgene is operably associated with an expression control sequence, under conditions whereby the transgene is expressed, whereby long chain
5 polyunsaturated fatty acid biosynthesis in the cells is altered.

The present invention is further directed toward pharmaceutical compositions comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

10 Relevant Literature

Production of gamma-linolenic acid by a $\Delta 6$ -desaturase is described in USPN 5,552,306 and USPN 5,614,393. Production of 8, 11-eicosadienoic acid using *Mortierella alpina* is disclosed in USPN 5,376,541. Production of docosahexaenoic acid by dinoflagellates is described in USPN 5,407,957.
15 Cloning of a $\Delta 6$ -desaturase from borage is described in PCT publication WO 96/21022. Cloning of $\Delta 9$ -desaturases is described in the published patent applications PCT WO 91/13972, EP 0 550 162 A1, EP 0 561 569 A2, EP 0 644 263 A2, and EP 0 736 598 A1, and in USPN 5,057,419. Cloning of $\Delta 12$ -desaturases from various organisms is described in PCT publication WO
20 94/11516 and USPN 5,443,974. Cloning of $\Delta 15$ -desaturases from various organisms is described in PCT publication WO 93/11245. A $\Delta 6$ palmitoyl-acyl carrier protein desaturase from *Thumbergia alata* and its expression in *E. coli* is described in USPN 5,614,400. Expression of a soybean stearyl-ACP desaturase in transgenic soybean embryos using a 35S promoter is disclosed in USPN
25 5,443,974.

SUMMARY OF THE INVENTION

Novel compositions and methods are provided for preparation of poly-unsaturated long chain fatty acids and desaturases in plants and plant cells. The methods involve growing a host plant cell of interest transformed with an
30 expression cassette functional in a host plant cell, the expression cassette

comprising a transcriptional and translational initiation regulatory region, joined in reading frame 5' to a DNA sequence encoding a desaturase polypeptide capable of modulating the production of PUFAs. Expression of the desaturase polypeptide provides for an alteration in the PUFA profile of host plant cells as a result of altered concentrations of enzymes involved in PUFA biosynthesis. Of particular interest is the selective control of PUFA production in plant tissues and/or plant parts such as leaves, roots, fruits and seeds. The invention finds use for example in the large scale production of DHA, EPA, ARA, and GLA and for modification of the fatty acid profile of edible plant tissues and/or plant parts.

The present invention further includes a purified nucleotide sequence or polypeptide sequence that is substantially related or homologous to the nucleotide and peptide sequences presented in SEQ ID NO:1 - SEQ ID NO:52. The present invention is further directed to methods of using the sequences presented in SEQ ID NO:1 to SEQ ID NO:40 as probes to identify related sequences, as components of expression systems and as components of systems useful for producing transgenic oil.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows possible pathways for the synthesis of arachidonic acid (20:4 Δ 5, 8, 11, 14) and stearidonic acid (18:4 Δ 6, 9, 12, 15) from palmitic acid (C_{16}) from a variety of organisms, including algae, *Mortierella* and humans. These PUFAs can serve as precursors to other molecules important for humans and other animals, including prostacyclins, leukotrienes, and prostaglandins, some of which are shown.

Figure 2 shows possible pathways for production of PUFAs in addition to ARA, including EPA and DHA, again compiled from a variety of organisms.

Figure 3A-E shows the DNA sequence (SEQ ID NO:1) of the *Mortierella alpina* Δ 6 desaturase and the deduced amino acid sequence (SEQ ID NO:2).

Figure 4 shows an alignment of the *Mortierella alpina* $\Delta 6$ desaturase amino acid sequence with other $\Delta 6$ desaturases and related sequences (SEQ ID NOS:7, 8, 9, 10, 11, 12 and 13).

Figure 5A-D shows the DNA sequence of the *Mortierella alpina* $\Delta 12$ desaturase (SEQ ID NO:3) and the deduced amino acid sequence (SEQ ID NO:4)

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:14) of the PCR fragment (see Example 1).

Figure 7A-D shows the DNA sequence of the *Mortierella alpina* $\Delta 5$ desaturase (SEQ ID NO:5).

Figure 8 shows alignments of the protein sequence of the $\Delta 5$ desaturase (SEQ ID NO:6) with $\Delta 6$ desaturases and related sequences (SEQ ID NOS:15, 16, 17, 18).

Figure 9 shows alignments of the protein sequence of the Ma 29 and contig 253538a.

Figure 10 shows alignments of the protein sequence of Ma 524 and contig 253538a.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

SEQ ID NO:1 shows the DNA sequence of the *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:2 shows the amino acid sequence of the *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:3 shows the DNA sequence of the *Mortierella alpina* $\Delta 12$ desaturase.

SEQ ID NO:4 shows the amino acid sequence of the *Mortierella alpina* $\Delta 12$ desaturase.

SEQ ID NO:5 shows the DNA sequence of the *Mortierella alpina* $\Delta 5$ desaturase.

SEQ ID NO:6 shows the amino acid sequence *Mortierella alpina* $\Delta 5$ desaturase.

5 SEQ ID NO:7 - SEQ ID NO:13 show amino acid sequences that relate to *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:14 shows an amino acid sequence of a PCR fragment of Example 1.

10 SEQ ID NO:15 - SEQ ID NO:18 show amino acid sequences that relate to *Mortierella alpina* $\Delta 5$ and $\Delta 6$ desaturases.

SEQ ID NO:19 - SEQ ID NO:30 show PCR primer sequences.

SEQ ID NO:31 - SEQ ID NO:37 show human nucleotide sequences.

SEQ ID NO:38 - SEQ ID NO:44 show human peptide sequences.

15 SEQ ID NO:45 - SEQ ID NO:46 show the nucleotide and amino acid sequence of a *Dictyostelium discoideum* desaturase.

SEQ ID NO:47 - SEQ ID NO:50 show the nucleotide and deduced amino acid sequence of a *Schizochytrium* cDNA clone.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 In order to ensure a complete understanding of the invention, the following definitions are provided:

$\Delta 5$ -Desaturase: $\Delta 5$ desaturase is an enzyme which introduces a double bond between carbons 5 and 6 from the carboxyl end of a fatty acid molecule.

$\Delta 6$ -Desaturase: $\Delta 6$ -desaturase is an enzyme which introduces a double bond between carbons 6 and 7 from the carboxyl end of a fatty acid molecule.

25 **$\Delta 9$ -Desaturase:** $\Delta 9$ -desaturase is an enzyme which introduces a double bond between carbons 9 and 10 from the carboxyl end of a fatty acid molecule.

$\Delta 12$ -Desaturase: $\Delta 12$ -desaturase is an enzyme which introduces a double bond between carbons 12 and 13 from the carboxyl end of a fatty acid molecule.

5 **Fatty Acids:** Fatty acids are a class of compounds containing a long hydrocarbon chain and a terminal carboxylate group. Fatty acids include the following:

| Fatty Acid | | |
|----------------------|--|--|
| 12:0 | lauric acid | |
| 16:0 | palmitic acid | |
| 16:1 | palmitoleic acid | |
| 18:0 | stearic acid | |
| 18:1 | oleic acid | $\Delta 9$ -18:1 |
| 18:2 $\Delta 5,9$ | taxoleic acid | $\Delta 5,9$ -18:2 |
| 18:2 $\Delta 6,9$ | 6,9-octadecadienoic acid | $\Delta 6,9$ -18:2 |
| 18:2 | linoleic acid | $\Delta 9,12$ -18:2 (LA) |
| 18:3 $\Delta 6,9,12$ | gamma-linolenic acid | $\Delta 6,9,12$ -18:3 (GLA) |
| 18:3 $\Delta 5,9,12$ | pinolenic acid | $\Delta 5,9,12$ -18:3 |
| 18:3 | alpha-linolenic acid | $\Delta 9,12,15$ -18:3 (ALA) |
| 18:4 | stearidonic acid | $\Delta 6,9,12,15$ -18:4 (SDA) |
| 20:0 | Arachidic acid | |
| 20:1 | Eicosenic Acid | |
| 22:0 | behehic acid | |
| 22:1 | erucic acid | |
| 22:2 | Docasadienoic acid | |
| 20:4 $\omega 6$ | arachidonic acid | $\Delta 5,8,11,14$ -20:4 (ARA) |
| 20:3 $\omega 6$ | $\omega 6$ -eicosatrienoic dihomogamma linolenic | $\Delta 8,11,14$ -20:3 (DGLA) |
| 20:5 $\omega 3$ | Eicosapentanoic (Timnodonic acid) | $\Delta 5,8,11,14,17$ -20:5 (EPA) |
| 20:3 $\omega 3$ | $\omega 3$ -eicosatrienoic | $\Delta 11,16,17$ -20:3 |
| 20:4 $\omega 3$ | $\omega 3$ -eicosatetraenoic | $\Delta 8,11,14,17$ -20:4 |
| 22:5 $\omega 3$ | Docosapentaenoic | $\Delta 7,10,13,16,19$ -22:5 ($\omega 3$ DPA) |
| 22:6 $\omega 3$ | Docosahexaenoic (cervonic acid) | $\Delta 4,7,10,13,16,19$ -22:6 (DHA) |
| 24:0 | Lignoceric acid | |

Taking into account these definitions, the present invention is directed to novel DNA sequences, DNA constructs, methods and compositions are provided which permit modification of the poly-unsaturated long chain fatty acid content of plant cells. Plant cells are transformed with an expression cassette

5 comprising a DNA encoding a polypeptide capable of increasing the amount of one or more PUFA in a plant cell. Desirably, integration constructs may be prepared which provide for integration of the expression cassette into the genome of a host cell. Host cells are manipulated to express a sense or antisense DNA encoding a polypeptide(s) that has desaturase activity. By

10 "desaturase" is intended a polypeptide which can desaturate one or more fatty acids to produce a mono- or poly-unsaturated fatty acid or precursor thereof of interest. By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification, for example, glycosylation or phosphorylation. The substrate(s) for the expressed enzyme may be produced

15 by the host cell or may be exogenously supplied.

To achieve expression in a host cell, the transformed DNA is operably associated with transcriptional and translational initiation and termination regulatory regions that are functional in the host cell. Constructs comprising the gene to be expressed can provide for integration into the genome of the host cell

20 or can autonomously replicate in the host cell. For production of linoleic acid (LA), the expression cassettes generally used include a cassette which provides for $\Delta 12$ desaturase activity, particularly in a host cell which produces or can take up oleic acid. For production of ALA, the expression cassettes generally used include a cassette which provides for $\Delta 15$ or $\omega 3$ desaturase activity,

25 particularly in a host cell which produces or can take up LA. For production of GLA or SDA, the expression cassettes generally used include a cassette which provides for $\Delta 6$ desaturase activity, particularly in a host cell which produces or can take up LA or ALA, respectively. Production of $\omega 6$ -type unsaturated fatty acids, such as LA or GLA, is favored in a plant capable of producing ALA by

30 inhibiting the activity of a $\Delta 15$ or $\omega 3$ type desaturase; this is accomplished by providing an expression cassette for an antisense $\Delta 15$ or $\omega 3$ transcript, or by

disrupting a $\Delta 15$ or $\omega 3$ desaturase gene. Similarly, production of LA or ALA is favored in a plant having $\Delta 6$ desaturase activity by providing an expression cassette for an antisense $\Delta 6$ transcript, or by disrupting a $\Delta 6$ desaturase gene. Production of oleic acid likewise is favored in a plant having $\Delta 12$ desaturase activity by providing an expression cassette for an antisense $\Delta 12$ transcript, or by disrupting a $\Delta 12$ desaturase gene. For production of ARA, the expression cassette generally used provides for $\Delta 5$ desaturase activity, particularly in a host cell which produces or can take up DGLA. Production of $\omega 6$ -type unsaturated fatty acids, such as ARA, is favored in a plant capable of producing ALA by inhibiting the activity of a $\Delta 15$ or $\omega 3$ type desaturase; this is accomplished by providing an expression cassette for an antisense $\Delta 15$ or $\omega 3$ transcript, or by disrupting a $\Delta 15$ or $\omega 3$ desaturase gene.

TRANSGENIC PLANT PRODUCTION OF FATTY ACIDS

Transgenic plant production of PUFAs offers several advantages over purification from natural sources such as fish or plants. Production of fatty acids from recombinant plants provides the ability to alter the naturally occurring plant fatty acid profile by providing new synthetic pathways in the host or by suppressing undesired pathways, thereby increasing levels of desired PUFAs, or conjugated forms thereof, and decreasing levels of undesired PUFAs. Production of fatty acids in transgenic plants also offers the advantage that expression of desaturase genes in particular tissues and/or plant parts means that greatly increased levels of desired PUFAs in those tissues and/or parts can be achieved, making recovery from those tissues more economical. For example, the desired PUFAs can be expressed in seed; methods of isolating seed oils are well established. In addition to providing a source for purification of desired PUFAs, seed oil components can be manipulated through expression of desaturase genes, either alone or in combination with other genes such as elongases, to provide seed oils having a particular PUFA profile in concentrated form. The concentrated seed oils then can be added to animal milks and/or synthetic or semi-synthetic milks to serve as infant formulas where human

nursing is impossible or undesired, or in cases of malnourishment or disease in both adults and infants.

For production of PUFAs, depending upon the host cell, the availability of substrate, and the desired end product(s), several polypeptides, particularly desaturases, are of interest including those polypeptides which catalyze the conversion of stearic acid to oleic acid, LA to GLA, of ALA to SDA, of oleic acid to LA, or of LA to ALA, which includes enzymes which desaturate at the $\Delta 6$, $\Delta 9$, $\Delta 12$, $\Delta 15$ or $\omega 3$ positions. Considerations for choosing a specific polypeptide having desaturase activity include the pH optimum of the polypeptide, whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired polyunsaturated fatty acid, and/or co-factors required by the polypeptide. The expressed polypeptide preferably has parameters compatible with the biochemical environment of its location in the host cell. For example, the polypeptide may have to compete for substrate with other enzymes in the host cell. Analyses of the K_m and specific activity of the polypeptide in question therefore are considered in determining the suitability of a given polypeptide for modifying PUFA production in a given host cell. The polypeptide used in a particular situation therefore is one which can function under the conditions present in the intended host cell but otherwise can be any polypeptide having desaturase activity which has the desired characteristic of being capable of modifying the relative production of a desired PUFA. A scheme for the synthesis of arachidonic acid (20:4 $\Delta 5$, 8, 11, 14) from palmitic acid (C_{16}) is shown in Figure 1. A key enzyme in this pathway is a $\Delta 5$ -desaturase which converts DH- γ -linolenic acid (DGLA, eicosatrienoic acid) to ARA. Conversion of α -linolenic acid (ALA) to stearidonic acid by a $\Delta 6$ -desaturase is also shown. Production of PUFAs in addition to ARA, including EPA and DHA is shown in Figure 2. A key enzyme in the synthesis of arachidonic acid (20:4 $\Delta 5$, 8, 11, 14) from stearic acid (C_{18}) is a $\Delta 6$ -desaturase which converts the linoleic acid into γ -linolenic acid. Conversion of α -linolenic acid (ALA) to stearidonic acid by a $\Delta 6$ -desaturase also is shown. For production of ARA, the DNA sequence

used encodes a polypeptide having $\Delta 5$ desaturase activity. In particular instances, this can be coupled with an expression cassette which provides for production of a polypeptide having $\Delta 6$ desaturase activity and, optionally, a transcription cassette providing for production of antisense sequences to a Δ 15 transcription product. The choice of combination of cassettes used depends in part on the PUFA profile of the host cell. Where the host cell $\Delta 5$ -desaturase activity is limiting, overexpression of $\Delta 5$ desaturase alone generally will be sufficient to provide for enhanced ARA production.

10 SOURCES OF POLYPEPTIDES HAVING DESATURASE ACTIVITY

As sources of polypeptides having desaturase activity and oligonucleotides encoding such polypeptides are organisms which produce a desired poly-unsaturated fatty acid. As an example, microorganisms having an ability to produce ARA can be used as a source of $\Delta 5$ -desaturase genes; 15 microorganisms which GLA or SDA can be used as a source of $\Delta 6$ -desaturase and/or $\Delta 12$ -desaturase genes. Such microorganisms include, for example, those belonging to the genera *Mortierella*, *Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor*, *Fusarium*, *Aspergillus*, *Rhodotorula*, and *Entomophthora*. Within the genus *Porphyridium*, of particular interest is *Porphyridium cruentum*. Within the genus *Mortierella*, of 20 particular interest are *Mortierella elongata*, *Mortierella exigua*, *Mortierella hygrophila*, *Mortierella ramanniana*, var. *angulispora*, and *Mortierella alpina*. Within the genus *Mucor*, of particular interest are *Mucor circinelloides* and *Mucor javanicus*.

25 DNAs encoding desired desaturases can be identified in a variety of ways. As an example, a source of the desired desaturase, for example genomic or cDNA libraries from *Mortierella*, is screened with detectable enzymatically- or chemically-synthesized probes, which can be made from DNA, RNA, or non-naturally occurring nucleotides, or mixtures thereof. Probes may be 30 enzymatically synthesized from DNAs of known desaturases for normal or

reduced-stringency hybridization methods. Oligonucleotide probes also can be used to screen sources and can be based on sequences of known desaturases, including sequences conserved among known desaturases, or on peptide sequences obtained from the desired purified protein. Oligonucleotide probes
5 based on amino acid sequences can be degenerate to encompass the degeneracy of the genetic code, or can be biased in favor of the preferred codons of the source organism. Oligonucleotides also can be used as primers for PCR from reverse transcribed mRNA from a known or suspected source; the PCR product can be the full length cDNA or can be used to generate a probe to obtain the
10 desired full length cDNA. Alternatively, a desired protein can be entirely sequenced and total synthesis of a DNA encoding that polypeptide performed.

Once the desired genomic or cDNA has been isolated, it can be sequenced by known methods. It is recognized in the art that such methods are subject to errors, such that multiple sequencing of the same region is routine and
15 is still expected to lead to measurable rates of mistakes in the resulting deduced sequence, particularly in regions having repeated domains, extensive secondary structure, or unusual base compositions, such as regions with high GC base content. When discrepancies arise, resequencing can be done and can employ special methods. Special methods can include altering sequencing conditions
20 by using: different temperatures; different enzymes; proteins which alter the ability of oligonucleotides to form higher order structures; altered nucleotides such as ITP or methylated dGTP; different gel compositions, for example adding formamide; different primers or primers located at different distances from the problem region; or different templates such as single stranded DNAs.
25 Sequencing of mRNA can also be employed.

For the most part, some or all of the coding sequence for the polypeptide having desaturase activity is from a natural source. In some situations, however, it is desirable to modify all or a portion of the codons, for example, to enhance expression, by employing host preferred codons. Host preferred
30 codons can be determined from the codons of highest frequency in the proteins expressed in the largest amount in a particular host species of interest. Thus, the

coding sequence for a polypeptide having desaturase activity can be synthesized in whole or in part. All or portions of the DNA also can be synthesized to remove any destabilizing sequences or regions of secondary structure which would be present in the transcribed mRNA. All or portions of the DNA also can be synthesized to alter the base composition to one more preferable in the desired host cell. Methods for synthesizing sequences and bringing sequences together are well established in the literature. *In vitro* mutagenesis and selection, site-directed mutagenesis, or other means can be employed to obtain mutations of naturally occurring desaturase genes to produce a polypeptide having desaturase activity *in vivo* with more desirable physical and kinetic parameters for function in the host cell, such as a longer half-life or a higher rate of production of a desired polyunsaturated fatty acid.

Desirable cDNAs have less than 60% A+T composition, preferably less than 50% A+T composition. On a localized scale of a sliding window of 20 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 75% A+T composition; with a window of 60 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 60%, more preferably no localized regions with greater than 55% A+T composition.

Mortierella alpina Desaturases

Of particular interest are the *Mortierella alpina* $\Delta 5$ -desaturase, $\Delta 6$ -desaturase and $\Delta 12$ -desaturase. The $\Delta 5$ -desaturase has 446 amino acids; the amino acid sequence is shown in Figure 7. The gene encoding the *Mortierella alpina* $\Delta 5$ -desaturase can be expressed in transgenic microorganisms to effect greater synthesis of ARA from DGLA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina* $\Delta 5$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina* $\Delta 5$ -desaturase polypeptide, also can be used. The *Mortierella alpina* $\Delta 6$ -desaturase, has 457 amino acids and a predicted molecular weight of 51.8 kD; the amino acid sequence is shown in Figure 3.

The gene encoding the *Mortierella alpina* $\Delta 6$ -desaturase can be expressed in transgenic plants or animals to effect greater synthesis of GLA from linoleic acid or of stearidonic acid (SDA) from ALA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina* $\Delta 6$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina* $\Delta 6$ -desaturase polypeptide, also can be used.

The *Mortierella alpina* $\Delta 12$ -desaturase has the amino acid sequence shown in Figure 5. The gene encoding the *Mortierella alpina* $\Delta 12$ -desaturase can be expressed in transgenic plants to effect greater synthesis of LA from oleic acid. Other DNAs which are substantially identical to the *Mortierella alpina* $\Delta 12$ -desaturase DNA, or which encode polypeptides which are substantially identical to the *Mortierella alpina* $\Delta 12$ -desaturase polypeptide, also can be used.

By substantially identical in sequence is intended an amino acid sequence or nucleic acid sequence exhibiting in order of increasing preference at least 60%, 80%, 90% or 95% homology to the *Mortierella alpina* $\Delta 5$ -desaturase amino acid sequence or nucleic acid sequence encoding the amino acid sequence. For polypeptides, the length of comparison sequences generally is at least 16 amino acids, preferably at least 20 amino acids, or most preferably 35 amino acids. For nucleic acids, the length of comparison sequences generally is at least 50 nucleotides, preferably at least 60 nucleotides, and more preferably at least 75 nucleotides, and most preferably, 110 nucleotides. Homology typically is measured using sequence analysis software, for example, the Sequence Analysis software package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wisconsin 53705, MEGAlign (DNASar, Inc., 1228 S. Park St., Madison, Wisconsin 53715), and MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, California 95008). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine and alanine;

valine, isoleucine and leucine; aspartic acid, glutamic acid, asparagine, and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Substitutions may also be made on the basis of conserved hydrophobicity or hydrophilicity (Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132, 1982), or on the basis of the ability to assume similar polypeptide secondary structure (Chou and Fasman, *Adv. Enzymol.* 47: 45-148, 1978).

Other Desaturases

Encompassed by the present invention are related desaturases from the same or other organisms. Such related desaturases include variants of the disclosed $\Delta 5$ -, $\Delta 6$ - and $\Delta 12$ -desaturases that occur naturally within the same or different species of *Mortierella*, as well as homologues of the disclosed $\Delta 5$ -desaturase from other species and evolutionarily related protein having desaturase activity. Also included are desaturases which, although not substantially identical to the *Mortierella alpina* $\Delta 5$ -desaturase, desaturate a fatty acid molecule at carbon 5, 6 or 12, respectively, from the carboxyl end of a fatty acid molecule. Related desaturases can be identified by their ability to function substantially the same as the disclosed desaturases; that is, are still able to effectively convert DGLA to ARA, LA to GLA, ALA to SDA or oleic acid to LA. Related desaturases also can be identified by screening sequence databases for sequences homologous to the disclosed desaturase, by hybridization of a probe based on the disclosed desaturase to a library constructed from the source organism, or by RT-PCR using mRNA from the source organism and primers based on the disclosed desaturase. Such desaturases includes those from humans, *Dictyostelium discoideum* and *Phaeodactylum tricornum*.

The regions of a desaturase polypeptide important for desaturase activity can be determined through routine mutagenesis, expression of the resulting mutant polypeptides and determination of their activities. Mutants may include deletions, insertions and point mutations, or combinations thereof. A typical functional analysis begins with deletion mutagenesis to determine the N- and C-terminal limits of the protein necessary for function, and then internal deletions,

insertions or point mutants are made to further determine regions necessary for function. Other techniques such as cassette mutagenesis or total synthesis also can be used. Deletion mutagenesis is accomplished, for example, by using exonucleases to sequentially remove the 5' or 3' coding regions. Kits are
5 available for such techniques. After deletion, the coding region is completed by ligating oligonucleotides containing start or stop codons to the deleted coding region after 5' or 3' deletion, respectively. Alternatively, oligonucleotides encoding start or stop codons are inserted into the coding region by a variety of methods including site-directed mutagenesis, mutagenic PCR or by ligation
10 onto DNA digested at existing restriction sites. Internal deletions can similarly be made through a variety of methods including the use of existing restriction sites in the DNA, by use of mutagenic primers via site directed mutagenesis or mutagenic PCR. Insertions are made through methods such as linker-scanning mutagenesis, site-directed mutagenesis or mutagenic PCR. Point mutations are
15 made through techniques such as site-directed mutagenesis or mutagenic PCR.

Chemical mutagenesis can also be used for identifying regions of a desaturase polypeptide important for activity. A mutated construct is expressed, and the ability of the resulting altered protein to function as a desaturase is assayed. Such structure-function analysis can determine which regions may be
20 deleted, which regions tolerate insertions, and which point mutations allow the mutant protein to function in substantially the same way as the native desaturase. All such mutant proteins and nucleotide sequences encoding them are within the scope of the present invention.

EXPRESSION OF DESATURASE GENES

25 Once the DNA encoding a desaturase polypeptide has been obtained, it is placed in a vector capable of replication in a host cell, or is propagated *in vitro* by means of techniques such as PCR or long PCR. Replicating vectors can include plasmids, phage, viruses, cosmids and the like. Desirable vectors include those useful for mutagenesis of the gene of interest or for expression of
30 the gene of interest in host cells. The technique of long PCR has made *in vitro* propagation of large constructs possible, so that modifications to the gene of

interest, such as mutagenesis or addition of expression signals, and propagation of the resulting constructs can occur entirely *in vitro* without the use of a replicating vector or a host cell.

For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is easily harvested, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location within the plant by using specific regulatory sequences, such as those of USPN 5,463,174, USPN 4,943,674, USPN 5,106,739, USPN 5,175,095, USPN 5,420,034, USPN 5,188,958, and USPN 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. In the present case, expression of desaturase genes, or antisense desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The $\Delta 5$ -desaturase polypeptide coding region is expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto *et al.*, PCT publication WO 95/24494). The termination region can be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected more as a matter of convenience rather than because of any particular property.

The choice of a host cell is influenced in part by the desired PUFA profile of the transgenic cell, and the native profile of the host cell. As an example, for production of linoleic acid from oleic acid, the DNA sequence used encodes a polypeptide having $\Delta 12$ desaturase activity, and for production of GLA from linoleic acid, the DNA sequence used encodes a polypeptide having $\Delta 6$ desaturase activity. Use of a host cell which expresses $\Delta 12$ desaturase activity and lacks or is depleted in $\Delta 15$ desaturase activity, can be used with an expression cassette which provides for overexpression of $\Delta 6$ desaturase alone generally is sufficient to provide for enhanced GLA production in the transgenic cell. Where the host cell expresses $\Delta 9$ desaturase activity, expression of both a $\Delta 12$ - and a $\Delta 6$ -desaturase can provide for enhanced GLA production. In particular instances where expression of $\Delta 6$ desaturase activity is coupled with expression of $\Delta 12$ desaturase activity, it is desirable that the host cell naturally have, or be mutated to have, low $\Delta 15$ desaturase activity. Alternatively, a host cell for $\Delta 6$ desaturase expression may have, or be mutated to have, high $\Delta 12$ desaturase activity.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, integration of constructs can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to

target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

When increased expression of the desaturase polypeptide in the source
5 plant is desired, several methods can be employed. Additional genes encoding the desaturase polypeptide can be introduced into the host organism. Expression from the native desaturase locus also can be increased through homologous recombination, for example by inserting a stronger promoter into the host genome to cause increased expression, by removing destabilizing
10 sequences from either the mRNA or the encoded protein by deleting that information from the host genome, or by adding stabilizing sequences to the mRNA (*see* USPN 4,910,141 and USPN 5,500,365.)

When it is desirable to express more than one different gene, appropriate regulatory regions and expression methods, introduced genes can be propagated
15 in the host cell through use of replicating vectors or by integration into the host genome. Where two or more genes are expressed from separate replicating vectors, it is desirable that each vector has a different means of replication. Each introduced construct, whether integrated or not, should have a different means of selection and should lack homology to the other constructs to maintain
20 stable expression and prevent reassortment of elements among constructs. Judicious choices of regulatory regions, selection means and method of propagation of the introduced construct can be experimentally determined so that all introduced genes are expressed at the necessary levels to provide for synthesis of the desired products.

25 Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. These techniques include transfection, infection, bolistic impact, electroporation, microinjection, scraping, or any other method which introduces the gene of interest into the host cell (*see* USPN 4,743,548, USPN 4,795,855, USPN 5,068,193, USPN 5,188,958, USPN 5,463,174, USPN
30 5,565,346 and USPN 5,565,347). For convenience, a host cell which has been manipulated by any method to take up a DNA sequence or construct will be

referred to as "transformed" or "recombinant" herein. The subject host will have at least have one copy of the expression construct and may have two or more, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy
5 numbers.

The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a separate marker construct may be introduced with the desired construct, as many transformation techniques introduce many DNA molecules into host cells. Typically,
10 transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene therefor may confer antibiotic resistance, or encode an essential growth factor or enzyme, and permit growth on selective media when
15 expressed in the transformed host cell. Desirably, resistance to kanamycin and the amino glycoside G418 are of interest (*see* USPN 5,034,322). Selection of a transformed host can also occur when the expressed marker protein can be detected, either directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by
20 its enzymatic activity; for example β galactosidase can convert the substrate X-gal to a colored product, and luciferase can convert luciferin to a light-emitting product. The marker protein can be detected by its light-producing or modifying characteristics; for example, the green fluorescent protein of *Aequorea victoria* fluoresces when illuminated with blue light. Antibodies can
25 be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as FACS or panning using antibodies.

The PUFAs produced using the subject methods and compositions may
30 be found in the host plant tissue and/or plant part as free fatty acids or in conjugated forms such as acylglycerols, phospholipids, sulfolipids or

glycolipids, and may be extracted from the host cell through a variety of means well-known in the art. Such means may include extraction with organic solvents, sonication, supercritical fluid extraction using for example carbon dioxide, and physical means such as presses, or combinations thereof. Of
5 particular interest is extraction with hexane or methanol and chloroform. Where desirable, the aqueous layer can be acidified to protonate negatively charged moieties and thereby increase partitioning of desired products into the organic layer. After extraction, the organic solvents can be removed by evaporation under a stream of nitrogen. When isolated in conjugated forms, the products are
10 enzymatically or chemically cleaved to release the free fatty acid or a less complex conjugate of interest, and are then subjected to further manipulations to produce a desired end product. Desirably, conjugated forms of fatty acids are cleaved with potassium hydroxide.

PURIFICATION OF FATTY ACIDS

15 If further purification is necessary, standard methods can be employed. Such methods include extraction, treatment with urea, fractional crystallization, HPLC, fractional distillation, silica gel chromatography, high speed centrifugation or distillation, or combinations of these techniques. Protection of reactive groups, such as the acid or alkenyl groups, may be done at any step
20 through known techniques, for example alkylation or iodination. Methods used include methylation of the fatty acids to produce methyl esters. Similarly, protecting groups may be removed at any step. Desirably, purification of fractions containing ARA, DHA and EPA is accomplished by treatment with urea and/or fractional distillation.

25 USES OF FATTY ACIDS

The uses of the fatty acids of subject invention are several. Probes based on the DNAs of the present invention may find use in methods for isolating related molecules or in methods to detect organisms expressing desaturases. When used as probes, the DNAs or oligonucleotides need to be detectable. This
30 is usually accomplished by attaching a label either at an internal site, for

example via incorporation of a modified residue, or at the 5' or 3' terminus. Such labels can be directly detectable, can bind to a secondary molecule that is detectably labeled, or can bind to an unlabelled secondary molecule and a detectably labeled tertiary molecule; this process can be extended as long as is practical to achieve a satisfactorily detectable signal without unacceptable levels of background signal. Secondary, tertiary, or bridging systems can include use of antibodies directed against any other molecule, including labels or other antibodies, or can involve any molecules which bind to each other, for example a biotin-streptavidin/avidin system. Detectable labels typically include radioactive isotopes, molecules which chemically or enzymatically produce or alter light, enzymes which produce detectable reaction products, magnetic molecules, fluorescent molecules or molecules whose fluorescence or light-emitting characteristics change upon binding. Examples of labelling methods can be found in USPN 5,011,770. Alternatively, the binding of target molecules can be directly detected by measuring the change in heat of solution on binding of probe to target via isothermal titration calorimetry, or by coating the probe or target on a surface and detecting the change in scattering of light from the surface produced by binding of target or probe, respectively, as may be done with the BIAcore system.

PUFAs of the subject invention produced by recombinant means find applications in a wide variety of areas. Supplementation of humans or animals with PUFAs in various forms can result in increased levels not only of the added PUFAs, but of their metabolic progeny as well. For example, where the inherent $\Delta 6$ -desaturase pathway is dysfunctional in an individual, treatment with GLA can result not only in increased levels of GLA, but also of downstream products such as ARA and prostaglandins (see Figure 1). Complex regulatory mechanisms can make it desirable to combine various PUFAs, or to add different conjugates of PUFAs, in order to prevent, control or overcome such mechanisms to achieve the desired levels of specific PUFAs in an individual.

PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary supplements, particularly in infant formulas, for patients

undergoing intravenous feeding or for preventing or treating malnutrition. Particular fatty acids such as EPA are used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. The predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 % as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to about 1.04 % as GLA. A preferred ratio of GLA:DGLA:ARA in infant formulas is from about 1:1:4 to about 1:1:1, respectively. Amounts of oils providing these ratios of PUFA can be determined without undue experimentation by one of skill in the art. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

NUTRITIONAL COMPOSITIONS

The present invention also includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced in accordance with the present invention and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to
5 glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey , electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to
10 the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present
15 invention will of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, and rehydration
20 compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

Nutritional Compositions

25 A typical nutritional composition of the present invention will contain edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amounts of such ingredients will vary depending on whether the formulation is intended for use with normal, healthy individuals temporarily exposed to stress, or to subjects having specialized needs due to certain chronic
30 or acute disease states (e.g., metabolic disorders). It will be understood by

persons skilled in the art that the components utilized in a nutritional formulation of the present invention are of semi-purified or purified origin. By semi-purified or purified is meant a material that has been prepared by purification of a natural material or by synthesis. These techniques are well known in the art (See, e.g., Code of Federal Regulations for Food Ingredients and Food Processing; Recommended Dietary Allowances, 10th Ed., National Academy Press, Washington, D.C., 1989).

In a preferred embodiment, a nutritional formulation of the present invention is an enteral nutritional product, more preferably an adult or child enteral nutritional product. Accordingly in a further aspect of the invention, a nutritional formulation is provided that is suitable for feeding adults or children who are experiencing stress. The formula comprises, in addition to the PUFAs of the invention; macronutrients, vitamins and minerals in amounts designed to provide the daily nutritional requirements of adults.

The macronutritional components include edible fats, carbohydrates and proteins. Exemplary edible fats are coconut oil, soy oil, and mono- and diglycerides and the PUFA oils of this invention. Exemplary carbohydrates are glucose, edible lactose and hydrolyzed cornstarch. A typical protein source would be soy protein, electrodialysed whey or electrodialysed skim milk or milk whey, or the hydrolysates of these proteins, although other protein sources are also available and may be used. These macronutrients would be added in the form of commonly accepted nutritional compounds in amount equivalent to those present in human milk or an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid and enteral nutritional formulas are well known in the art and are described in detail in the examples.

The enteral formula can be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or a powder. The powder can be prepared by spray drying the enteral formula prepared as indicated above, and the formula can be reconstituted by rehydrating the concentrate. Adult and infant nutritional formulas are well known in the art and commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum®

from Ross Products Division, Abbott Laboratories). An oil or acid of the present invention can be added to any of these formulas in the amounts described below.

5 The energy density of the nutritional composition when in liquid form, can typically range from about 0.6 Kcal to 3 Kcal per ml. When in solid or powdered form, the nutritional supplement can contain from about 1.2 to more than 9 Kcals per gm, preferably 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and more preferably less than 660 mOsm.

10 The nutritional formula would typically include vitamins and minerals, in addition to the PUFAs of the invention, in order to help the individual ingest the minimum daily requirements for these substances. In addition to the PUFAs listed above, it may also be desirable to supplement the nutritional composition with zinc, copper, and folic acid in addition to antioxidants. It is believed that
15 these substances will also provide a boost to the stressed immune system and thus will provide further benefits to the individual. The presence of zinc, copper or folic acid is optional and is not required in order to gain the beneficial effects on immune suppression. Likewise a pharmaceutical composition can be supplemented with these same substances as well.

20 In a more preferred embodiment, the nutritional contains, in addition to the antioxidant system and the PUFA component, a source of carbohydrate wherein at least 5 weight % of said carbohydrate is an indigestible oligosaccharide. In yet a more preferred embodiment, the nutritional composition additionally contains protein, taurine and carnitine.

25 The PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary substitutes, or supplements, particularly infant formulas, for patients undergoing intravenous feeding or for preventing or treating malnutrition. Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 %
30 as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to about 1.04 % as GLA.

Additionally, the predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Thus, fatty acids such as ARA, DGLA, GLA and/or EPA produced by the invention can be used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. In particular, an oil composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of ARA, DGLA and GLA. More preferably the oil will comprise from about 0.3 to 30% ARA, from about 0.2 to 30% DGLA, and from about 0.2 to about 30% GLA.

In addition to the concentration, the ratios of ARA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, an oil composition which contains two or more of ARA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of ARA:DGLA:DGL ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to ARA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to ARA can be used to produce an ARA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an ARA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of desaturase expression as described can be used to modulate the PUFA levels and ratios. Depending on the expression system used, e.g., cell culture or an animal expressing oil(s) in its milk, the oils also can be isolated and recombined in the desired concentrations and ratios. Amounts of oils providing these ratios of PUFA can be determined following standard protocols. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

For dietary supplementation, the purified PUFAs, or derivatives thereof, may be incorporated into cooking oils, fats or margarines formulated so that in normal use the recipient would receive the desired amount. The PUFAs may also be incorporated into infant formulas, nutritional supplements or other food products, and may find use as anti-inflammatory or cholesterol lowering agents.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream.

Possible routes of administration include, for example, oral, rectal and parenteral. The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Pharmaceutical compositions may be utilized to administer the PUFA component to an individual. Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile solutions or dispersions for ingestion. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances, and the like.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs of the invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with the antioxidants and the PUFA component. The amount of the antioxidants and PUFA component that should

be incorporated into the pharmaceutical formulation should fit within the guidelines discussed above.

As used in this application, the term "treat" refers to either preventing, or reducing the incidence of, the undesired occurrence. For example, to treat
5 immune suppression refers to either preventing the occurrence of this suppression or reducing the amount of such suppression. The terms "patient" and "individual" are being used interchangeably and both refer to an animal. The term "animal" as used in this application refers to any warm-blooded mammal including, but not limited to, dogs, humans, monkeys, and apes. As
10 used in the application the term "about" refers to an amount varying from the stated range or number by a reasonable amount depending upon the context of use. Any numerical number or range specified in the specification should be considered to be modified by the term about.

"Dose" and "serving" are used interchangeably and refer to the amount
15 of the nutritional or pharmaceutical composition ingested by the patient in a single setting and designed to deliver effective amounts of the antioxidants and the structured triglyceride. As will be readily apparent to those skilled in the art, a single dose or serving of the liquid nutritional powder should supply the amount of antioxidants and PUFAs discussed above. The amount of the dose or
20 serving should be a volume that a typical adult can consume in one sitting. This amount can vary widely depending upon the age, weight, sex or medical condition of the patient. However as a general guideline, a single serving or dose of a liquid nutritional produce should be considered as encompassing a volume from 100 to 600 ml, more preferably from 125 to 500 ml and most
25 preferably from 125 to 300 ml.

The PUFAs of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils,
30 cooking oils, cooking fats, meats, fish and beverages.

Pharmaceutical Applications

For pharmaceutical use (human or veterinary), the compositions are generally administered orally but can be administered by any route by which they may be successfully absorbed, e.g., parenterally (i.e. subcutaneously, intramuscularly or intravenously), rectally or vaginally or topically, for example, as a skin ointment or lotion. The PUFAs of the present invention may be administered alone or in combination with a pharmaceutically acceptable carrier or excipient. Where available, gelatin capsules are the preferred form of oral administration. Dietary supplementation as set forth above also can provide an oral route of administration. The unsaturated acids of the present invention may be administered in conjugated forms, or as salts, esters, amides or prodrugs of the fatty acids. Any pharmaceutically acceptable salt is encompassed by the present invention; especially preferred are the sodium, potassium or lithium salts. Also encompassed are the N-alkylpolyhydroxamine salts, such as N-methyl glucamine, found in PCT publication WO 96/33155. The preferred esters are the ethyl esters. As solid salts, the PUFAs also can be administered in tablet form. For intravenous administration, the PUFAs or derivatives thereof may be incorporated into commercial formulations such as Intralipids. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of ARA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered, either alone or in mixtures with other PUFAs, to achieve a normal fatty acid profile in a patient. Where desired, the individual components of formulations may be individually provided in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g, or even 100 g daily, and is preferably from 10 mg to 1, 2, 5 or 10 g daily as required, or molar equivalent amounts of derivative forms thereof. Parenteral nutrition compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention; preferred is a composition having from about 1 to about 25 weight percent of the total PUFA composition as GLA (USPN 5,196,198). Other vitamins, and particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. Where desired, a

preservative such as α tocopherol may be added, typically at about 0.1% by weight.

Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectible solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglyol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances and the like.

An especially preferred pharmaceutical composition contains diacetyltartaric acid esters of mono- and diglycerides dissolved in an aqueous medium or solvent. Diacetyltartaric acid esters of mono- and diglycerides have an HLB value of about 9-12 and are significantly more hydrophilic than existing antimicrobial lipids that have HLB values of 2-4. Those existing hydrophobic lipids cannot be formulated into aqueous compositions. As disclosed herein, those lipids can now be solubilized into aqueous media in combination with diacetyltartaric acid esters of mono- and diglycerides. In accordance with this embodiment, diacetyltartaric acid esters of mono- and diglycerides (e.g., DATEM-C12:0) is melted with other active antimicrobial lipids (e.g., 18:2 and 12:0 monoglycerides) and mixed to obtain a homogeneous mixture.

Homogeneity allows for increased antimicrobial activity. The mixture can be completely dispersed in water. This is not possible without the addition of diacetyltartaric acid esters of mono- and diglycerides and premixing with other monoglycerides prior to introduction into water. The aqueous composition can
5 then be admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants as may be required to form a spray or inhalant.

The present invention also encompasses the treatment of numerous disorders with fatty acids. Supplementation with PUFAs of the present
10 invention can be used to treat restenosis after angioplasty. Symptoms of inflammation, rheumatoid arthritis, and asthma and psoriasis can be treated with the PUFAs of the present invention. Evidence indicates that PUFAs may be involved in calcium metabolism, suggesting that PUFAs of the present invention may be used in the treatment or prevention of osteoporosis and of
15 kidney or urinary tract stones.

The PUFAs of the present invention can be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions; addition of fatty acids has been shown to slow their growth and cause cell death, and to increase their susceptibility to chemotherapeutic agents.
20 GLA has been shown to cause reexpression on cancer cells of the E-cadherin cellular adhesion molecules, loss of which is associated with aggressive metastasis. Clinical testing of intravenous administration of the water soluble lithium salt of GLA to pancreatic cancer patients produced statistically significant increases in their survival. PUFA supplementation may also be
25 useful for treating cachexia associated with cancer.

The PUFAs of the present invention can also be used to treat diabetes (USPN 4,826,877; Horrobin *et al.*, Am. J. Clin. Nutr. Vol. 57 (Suppl.), 732S-737S). Altered fatty acid metabolism and composition has been demonstrated in diabetic animals. These alterations have been suggested to be involved in
30 some of the long-term complications resulting from diabetes, including retinopathy, neuropathy, nephropathy and reproductive system damage.

Primrose oil, which contains GLA, has been shown to prevent and reverse diabetic nerve damage.

The PUFAs of the present invention can be used to treat eczema, reduce blood pressure and improve math scores. Essential fatty acid deficiency has
5 been suggested as being involved in eczema, and studies have shown beneficial effects on eczema from treatment with GLA. GLA has also been shown to reduce increases in blood pressure associated with stress, and to improve performance on arithmetic tests. GLA and DGLA have been shown to inhibit
10 platelet aggregation, cause vasodilation, lower cholesterol levels and inhibit proliferation of vessel wall smooth muscle and fibrous tissue (Brenner *et al.*, Adv. Exp. Med. Biol. Vol. 83, p. 85-101, 1976). Administration of GLA or DGLA, alone or in combination with EPA, has been shown to reduce or prevent gastro-intestinal bleeding and other side effects caused by non-steroidal anti-inflammatory drugs (USPN 4,666,701). GLA and DGLA have also been shown
15 to prevent or treat endometriosis and premenstrual syndrome (USPN 4,758,592) and to treat myalgic encephalomyelitis and chronic fatigue after viral infections (USPN 5,116,871).

Further uses of the PUFAs of this invention include use in treatment of AIDS, multiple sclerosis, acute respiratory syndrome, hypertension and
20 inflammatory skin disorders. The PUFAs of the inventions also can be used for formulas for general health as well as for geriatric treatments.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals, as well as
25 humans, as animals experience many of the same needs and conditions as human. For example, the oil or acids of the present invention may be utilized in animal feed supplements or as animal feed substitutes.

The following examples are presented by way of illustration, not of limitation.

Examples

- 5
Example 1 Isolation of $\Delta 5$ Desaturase Nucleotide Sequence from *Mortierella alpina*
- Example 2 Isolation of $\Delta 6$ Desaturase Nucleotide Sequence from *Mortierella alpina*
- Example 3 Identification of $\Delta 6$ Desaturases Homologues to the *Mortierella alpina* Δ Desaturase
- Example 4 Isolation of D-12 Desaturase Nucleotide Sequence from *Mortierella alpina*
- 10
Example 5 Isolation of Cytochrome b5 Reductase Nucleotide Sequence from *Mortierella alpina*
- Example 6 Expression of *M. alpina* Desaturase Clones in Baker's Yeast
- 15
Example 7 Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants
- Example 8 Expression of *M. alpina* $\Delta 6$ Desaturase in *Brassica napus*
- 20
Example 9 Expression of *M. alpina* $\Delta 12$ desaturase in *Brassica napus*
- Example 10 Simultaneous expression of *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*
- 25
Example 11 Simultaneous expression of *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases in *Brassica napus*
- Example 12 Simultaneous expression of *M. alpina* $\Delta 5$, $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*
- Example 13 Stereospecific Distribution of $\Delta 6$ -Desaturated Oils
- Example 14 Fatty Acid Compositions of Transgenic Plants

Example 15 Combined Expression of $\Delta 6$ and $\Delta 12$ Desaturases in *B. napus* Achieved by Crossing

Example 16 Expression of *M. alpina* desaturases in soybean

Example 17 Human Desaturase Gene Sequences

5

Example 1

Isolation of a $\Delta 5$ -desaturase Nucleotide Sequence from *Mortierella alpina*

Mortierella alpina produces arachidonic acid (ARA, 20:4) from the precursor 20:3 by a $\Delta 5$ -desaturase. A nucleotide sequence encoding the $\Delta 5$ -desaturase from *Mortierella alpina* (see Figure 7) was obtained through PCR
10 amplification using *M. alpina* 1st strand cDNA and degenerate oligonucleotide primers corresponding to amino acid sequences conserved between $\Delta 6$ -desaturases from *Synechocystis* and *Spirulina*. The procedure used was as follows:

Total RNA was isolated from a 3 day old PUFA-producing culture of
15 *Mortierella alpina* using the protocol of Hoge *et al.* (1982) *Experimental Mycology* 6:225-232. The RNA was used to prepare double-stranded cDNA using BRL's lambda-ZipLox system, following the manufacturer's instructions. Several size fractions of the *M. alpina* cDNA were packaged separately to yield libraries with different average-sized inserts. The "full-length" library contains
20 approximately 3×10^6 clones with an average insert size of 1.77 kb. The "sequencing-grade" library contains approximately 6×10^5 clones with an average insert size of 1.1 kb.

5 μ g of total RNA was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTTTT-
25 3' (SEQ ID NO:19.) Degenerate oligonucleotides were designed to regions conserved between the two cyanobacterial $\Delta 6$ -desaturase sequences. The specific primers used were:

D6DESAT-F3 (SEQ ID NO:20)

5'-CUACUACUACUACAYCAYACOTAYACOAAYAT-3'

D6DESAT-R3 (SEQ ID NO:21)

5'-CAUCAUCAUCAUOGGAAOARRTGRTG-3'

- 5 where Y=C+T, R=A+G, and O=I+C. PCR amplification was carried out in a 25 µl volume containing: template derived from 40 ng total RNA, 2 pM each primer, 200 µM each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH₄)₂SO₄, 2 mM MgCl₂. Samples were subjected to an initial desaturation step of 95 degrees (all temperatures Celsius) for 5 minutes, then
- 10 held at 72 degrees while 0.2 U of Taq polymerase were added. PCR thermocycling conditions were as follows: 94 degrees for 1 min., 45 degrees for 1.5 min., 72 degrees for 2 min. PCR was continued for 35 cycles. PCR using these primers on the *M. alpina* first-strand cDNA produced a 550 bp reaction product. Comparison of the deduced amino acid sequence of the *M.*
- 15 *alpina* PCR fragment revealed regions of homology with Δ6-desaturases (see Figure 4). However, there was only about 28% identity over the region compared. The deduced amino acid sequence is presented in SEQ ID NO:14.

- The PCR product was used as a probe to isolate corresponding cDNA clones from a *M. alpina* library. The longest cDNA clone, Ma29, was
- 20 designated pCGN5521 and has been completely sequenced on both strands. The cDNA is contained as a 1481 bp insert in the vector pZL1 (Bethesda Research Laboratories) and, beginning with the first ATG, contains an open reading frame encoding 446 amino acids. The reading frame contains the sequence deduced from the PCR fragment. The sequence of the cDNA insert
- 25 was found to contain regions of homology to Δ6-desaturases (see Figure 8). For example, three conserved "histidine boxes" (that have been observed in other membrane-bound desaturases (Okuley *et al.*, (1994) *The Plant Cell* 6:147-158)) were found to be present in the *Mortierella* sequence at amino acid positions 171-175, 207-212, and 387-391 (see Figure 5A-5D). However, the typical
- 30 "HXXHH" amino acid motif for the third histidine box for the *Mortierella*

desaturase was found to be QXXHH. The amino-terminus of the encoded protein, showed significant homology to cytochrome b5 proteins. Thus, the *Mortierella* cDNA clone appears to represent a fusion between a cytochrome b5 and a fatty acid desaturase. Since cytochrome b5 is believed to function as the electron donor for membrane-bound desaturase enzymes, it is possible that the N-terminal cytochrome b5 domain of this desaturase protein is involved in its function. This may be advantageous when expressing the desaturase in heterologous systems for PUFA production.

Example 2

10 Isolation of $\Delta 6$ Desaturase Nucleotide Sequence from *Mortierella alpina*

A nucleic acid sequence from a partial cDNA clone, Ma524, encoding a $\Delta 6$ fatty acid desaturase from *Mortierella alpina* was obtained by random sequencing of clones from the *M. alpina* cDNA library described in Example 1. cDNA-containing plasmids were excised as follows:

15 Five μ l of phage were combined with 100 μ l of *E. coli* DH10B(ZIP) grown in ECLB plus 10 μ g/ml kanamycin, 0.2% maltose, and 10 mM $MgSO_4$ and incubated at 37 degrees for 15 minutes. 0.9 ml SOC was added and 100 μ l of the bacteria immediately plated on each of 10 ECLB + 50 μ g Pen plates. No 45 minute recovery time was needed. The plates were incubated overnight at 37
20 degrees. Colonies were picked into ECLB + 50 μ g Pen media for overnight cultures to be used for making glycerol stocks and miniprep DNA. An aliquot of the culture used for the miniprep is stored as a glycerol stock. Plating on ECLB + 50 μ g Pen/ml resulted in more colonies and a greater proportion of colonies containing inserts than plating on 100 μ g/ml Pen.

25 Random colonies were picked and plasmid DNA purified using Qiagen miniprep kits. DNA sequence was obtained from the 5' end of the cDNA insert and compared to the databases using the BLAST algorithm. Ma524 was identified as a putative $\Delta 6$ desaturase based on DNA sequence homology to previously identified $\Delta 6$ desaturases. A full-length cDNA clone was isolated

from the *M. alpina* library. The abundance of this clone appears to be slightly (2X) less than Ma29. Ma524 displays significant homology to a portion of a *Caenorhabditis elegans* cosmid, WO6D2.4, a cytochrome b5/desaturase fusion protein from sunflower, and the two $\Delta 6$ desaturases in the public databanks
5 those from *Synechocystis* and *Spirulina*.

In addition, Ma524 shows significant homology to the borage $\Delta 6$ -desaturase sequence (PCT publication WO 96/21022). Ma524 thus appears to encode a $\Delta 6$ -desaturase that is related to the borage and algal $\Delta 6$ -desaturases. It should be noted that, although the amino acid sequences of Ma524 and the
10 borage $\Delta 6$ are similar, the base composition of the cDNAs is quite different: the borage cDNA has an overall base composition of 60 % A+T, with some regions exceeding 70 %, while Ma524 has an average of 44 % A+T base composition, with no regions exceeding 60 %. This may have implications for expressing the cDNAs in microorganisms or animals which favor different base compositions.
15 It is known that poor expression of recombinant genes can occur when the host has a very different base composition from that of the introduced gene. Speculated mechanisms for such poor expression include decreased stability or translatability of the mRNA.

Example 3

20 Identification of $\Delta 6$ -desaturases Homologous to the *Mortierella alpina* $\Delta 6$ -desaturase

Nucleic acid sequences that encode putative $\Delta 6$ -desaturases were identified through a BLASTX search of the est databases through NCBI using the Ma524 amino acid sequence. Several sequences showed significant
25 homology. In particular, the deduced amino acid sequence of two *Arabidopsis thaliana* sequences, (accession numbers F13728 and T42806) showed homology to two different regions of the deduced amino acid sequence of Ma524. The following PCR primers were designed: ATTS4723-FOR (complementary to F13728) 5'-CUACUACUACUAGGAGTCCTCTA
30 CGGTGTTTTG, SEQ ID NO:22, and T42806-REV (complementary to

T42806) 5' CAUCAUCAUATGATGCTCAAGCTGAACTG, SEQ ID NO:23. Five µg of total RNA isolated from developing siliques of *Arabidopsis thaliana* was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CCAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTTTT-3', (SEQ ID NO:24). PCR was carried out in a 50 µl volume containing: template derived from 25 ng total RNA, 2 pM each primer, 200 µM each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.2 U Taq Polymerase. Cycle conditions were as follows: 94 degrees for 30 sec., 50 degrees for 30 sec., 72 degrees for 30 sec. PCR was continued for 35 cycles followed by an additional extension at 72 degrees for 7 minutes. PCR resulted in a fragment of ~750 base pairs which was subsequently subcloned, named 12-5, and sequenced. Each end of this fragment corresponds to the *Arabidopsis* est from which the PCR primers were derived. This is the sequence named 12-5. The deduced amino acid sequence of 12-5 is compared to that of Ma524 and ests from human (W28140), mouse (W53753), and *C. elegans* (R05219) in Figure 4. Based on homology, these sequences represent desaturase polypeptides. The full-length genes can be cloned using probes based on the est sequences. The genes can then be placed in expression vectors and expressed in host cells and their specific Δ⁶- or other desaturase activity can be determined as described below.

Example 4

Isolation of Δ¹² Desaturase Nucleotide Sequence from *Mortierella alpina*

Based on the fatty acids it accumulates, *Mortierella alpina* has an ω₆ type desaturase. The ω₆ desaturase is responsible for the production of linoleic acid (18:2) from oleic acid (18:1). Linoleic acid (18:2) is a substrate for a Δ⁶ desaturase. This experiment was designed to determine if *Mortierella alpina* has a Δ¹²-desaturase polypeptide, and if so, to identify the corresponding nucleotide sequence. A random colony from the *M. alpina* sequencing grade library, Ma648, was sequenced and identified as a putative desaturase based on DNA sequence homology to previously identified desaturases, as described for

Ma524 (*see* Example 2). The deduced amino acid sequence from the 5' end of the Ma648 cDNA displays significant homology to soybean microsomal ω 6 (Δ 12) desaturase (accession #L43921) as well as castor bean oleate 12-hydroxylase (accession #U22378). In addition, homology is observed to a variety of other ω 6 (Δ 12) and ω 3 (Δ 15) fatty acid desaturase sequences.

Example 5

Isolation of Cytochrome b5 Reductase Nucleotide Sequence from *Mortierella alpina*

A nucleic acid sequence encoding a cytochrome b5 reductase from *Mortierella alpina* was obtained as follows. A cDNA library was constructed based on total RNA isolated from *Mortierella alpina* as described in Example 1. DNA sequence was obtained from the 5' and 3' ends of one of the clones, M12-27. A search of public databanks with the deduced amino acid sequence of the 3' end of M12-27 (*see* Figure 5) revealed significant homology to known cytochrome b5 reductase sequences. Specifically, over a 49 amino acid region, the *Mortierella* clone shares 55% identity (73% homology) with a cytochrome b5 reductase from pig (*see* Figure 4).

Example 6

Expression of *M. alpina* Desaturase Clones in Baker's Yeast **Yeast Transformation**

Lithium acetate transformation of yeast was performed according to standard protocols (*Methods in Enzymology*, Vol. 194, p. 186-187, 1991). Briefly, yeast were grown in YPD at 30°C. Cells were spun down, resuspended in TE, spun down again, resuspended in TE containing 100 mM lithium acetate, spun down again, and resuspended in TE/lithium acetate. The resuspended yeast were incubated at 30°C for 60 minutes with shaking. Carrier DNA was added, and the yeast were aliquoted into tubes. Transforming DNA was added, and the tubes were incubated for 30 min. at 30°C. PEG solution (35% (w/v) PEG 4000, 100 mM lithium acetate, TE pH7.5) was added followed by a 50

min. incubation at 30°C. A 5 min. heat shock at 42°C was performed, the cells were pelleted, washed with TE, pelleted again and resuspended in TE. The resuspended cells were then plated on selective media.

Desaturase Expression in Transformed Yeast

5 cDNA clones from *Mortierella alpina* were screened for desaturase activity in baker's yeast. A canola $\Delta 15$ -desaturase (obtained by PCR using 1st strand cDNA from *Brassica napus* cultivar 212/86 seeds using primers based on the published sequence (Arondel *et al. Science* 258:1353-1355)) was used as a positive control. The $\Delta 15$ -desaturase gene and the gene from cDNA clone
10 Ma29 was put in the expression vector pYES2 (Invitrogen), resulting in plasmids pCGR-2 and pCGR-4, respectively. These plasmids were transfected into *S. cerevisiae* yeast strain 334 and expressed after induction with galactose and in the presence of substrates that allowed detection of specific desaturase activity. The control strain was *S. cerevisiae* strain 334 containing the unaltered
15 pYES2 vector. The substrates used, the products produced and the indicated desaturase activity were: DGLA (conversion to ARA would indicate $\Delta 5$ -desaturase activity), linoleic acid (conversion to GLA would indicate $\Delta 6$ -desaturase activity; conversion to ALA would indicate $\Delta 15$ -desaturase activity), oleic acid (an endogenous substrate made by *S. cerevisiae*, conversion to
20 linoleic acid would indicate $\Delta 12$ -desaturase activity, which *S. cerevisiae* lacks), or ARA (conversion to EPA would indicate $\Delta 17$ -desaturase activity). The results are provided in Table 1 below. The lipid fractions were extracted as follows: Cultures were grown for 48-52 hours at 15°C. Cells were pelleted by centrifugation, washed once with sterile ddH₂O, and repelleted. Pellets were
25 vortexed with methanol; chloroform was added along with tritridecanoin (as an internal standard). The mixtures were incubated for at least one hour at room temperature or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated
30 at 40°C under a stream of nitrogen. The extracted lipids were then derivatized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by

adding 2 ml of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C to 100°C for 30 minutes and cooled to room temperature. Approximately 2 ml of 14 % boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml
5 of water and 1 ml of hexane were added to extract the FAME for analysis by GC. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate added) and then multiplying by 100. To calculate the oleic acid percent conversion, as no substrate was added, the total linoleic acid produced was divided by the sum of
10 (oleic acid and linoleic acid produced), then multiplying by 100.

Table 1***M. alpina* Desaturase Expression in Baker's Yeast**

| CLONE | TYPE OF ENZYME ACTIVITY | % CONVERSION OF SUBSTRATE |
|------------------------------------|----------------------------|--------------------------------|
| pCGR-2 | $\Delta 6$ | 0 (18:2 to 18:3 ω 6) |
| (canola $\Delta 15$ desaturase) | $\Delta 15$ | 16.3 (18:2 to 18:3 ω 3) |
| | $\Delta 5$ | 2.0 (20:3 to 20:4 ω 6) |
| | $\Delta 17$ | 2.8 (20:4 to 20:5 ω 3) |
| | $\Delta 12$ | 1.8 (18:1 to 18:2 ω 6) |
| pCGR-4 | $\Delta 6$ | 0 |
| (M. alpina | $\Delta 15$ | 0 |
| $\Delta 6$ -like, Ma29) | $\Delta 5$ | 15.3 |
| | $\Delta 17$ | 0.3 |
| | $\Delta 12$ | 3.3 |
| pCGR-7 | $\Delta 6$ | 0 |
| (M. alpina | $\Delta 15$ | 3.8 |
| $\Delta 12$ -like, Ma648 | $\Delta 5$ | 2.2 |
| | $\Delta 17$ | 0 |
| | $\Delta 12$ | 63.4 |

The $\Delta 15$ -desaturase control clone exhibited 16.3% conversion of the
 5 substrate. The pCGR-4 clone expressing the Ma29 cDNA converted 15.3% of
 the 20:3 substrate to 20:4 ω 6, indicating that the gene encodes a $\Delta 5$ -desaturase.
 The background (non-specific conversion of substrate) was between 0-3% in
 these cases. The pCGR-5 clone expressing the Ma524 cDNA showed 6%
 conversion of the substrate to GLA, indicating that the gene encodes a $\Delta 6$ -
 10 desaturase. The pCGR-7 clone expressing the Ma648 cDNA converted 63.4%
 conversion of the substrate to LA, indicating that the gene encodes a $\Delta 12$ -
 desaturase. Substrate inhibition of activity was observed by using different
 concentrations of the substrate. When substrate was added to 100 μ M, the
 percent conversion to product dropped as compared to when substrate was
 15 added to 25 μ M (see below). These data show that desaturases with different

substrate specificities can be expressed in a heterologous system and used to produce PUFAs.

Table 2 represents fatty acids of interest as a percent of the total lipid extracted from the yeast host *S. cerevisiae* 334 with the indicated plasmid. No
5 glucose was present in the growth media. Affinity gas chromatography was used to separate the respective lipids. GC/MS was employed to verify the identity of the product(s). The expected product for the *B. napus* $\Delta 15$ -desaturase, α -linolenic acid, was detected when its substrate, linoleic acid, was added exogenously to the induced yeast culture. This finding demonstrates that
10 yeast expression of a desaturase gene can produce functional enzyme and detectable amounts of product under the current growth conditions. Both exogenously added substrates were taken up by yeast, although slightly less of the longer chain PUFA, dihomo- γ -linolenic acid (20:3), was incorporated into yeast than linoleic acid (18:2) when either was added in free form to the induced
15 yeast cultures. γ -linolenic acid was detected when linoleic acid was present during induction and expression of *S. cerevisiae* 334 (pCGR-5). The presence of this PUFA demonstrates $\Delta 6$ -desaturase activity from pCGR-5 (MA524). Linoleic acid, identified in the extracted lipids from expression of *S. cerevisiae* 334 (pCGR-7), classifies the cDNA MA648 from *M. alpina* as the $\Delta 12$ -
20 desaturase.

Table 2
Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

| Plasmid in Yeast (enzyme) | 18:2 Incorporated | α -18:3 Produced | γ -18:3 Produced | 20:3 Incorporated | 20:4 Produced | 18:1* Present | 18:2 Produced |
|---------------------------------|----------------------|----------------------------|----------------------------|----------------------|------------------|------------------|------------------|
| pYES2 (control) | 66.9 | 0 | 0 | 58.4 | 0 | 4 | 0 |
| pCGR-2 ($\Delta 15$) | 60.1 | 5.7 | 0 | 50.4 | 0 | 0.7 | 0 |
| pCGR-4 ($\Delta 5$) | 67 | 0 | 0 | 32.3 | 5.8 | 0.8 | 0 |
| pCGR-5 ($\Delta 6$) | 62.4 | 0 | 4.0 | 49.9 | 0 | 2.4 | 0 |
| pCGR-7 ($\Delta 12$) | 65.6 | 0 | 0 | 45.7 | 0 | 7.1 | 12.2 |

100 μ M substrate added

* 18:1 is an endogenous fatty acid in yeast

- 5 Key To Tables
18:1 =oleic acid
18:2 =linoleic acid
 α -18:3 = α -linolenic acid
 γ -18:3 = γ -linolenic acid
18:4 =stearidonic acid
20:3 =dihomo- γ -linolenic acid
20:4 =arachidonic acid

Example 7

Expression of $\Delta 5$ Desaturase in Plants

Expression in Leaves

5 This experiment was designed to determine whether leaves expressing Ma29 (as determined by Northern) were able to convert exogenously applied DGLA (20:3) to ARA (20:4).

The Ma29 desaturase cDNA was modified by PCR to introduce convenient restriction sites for cloning. The desaturase coding region has been inserted into a d35 cassette under the control of the double 35S promoter for
10 expression in *Brassica* leaves (pCGN5525) following standard protocols (*see* USPN 5,424,200 and USPN 5,106,739). Transgenic *Brassica* plants containing pCGN5525 were generated following standard protocols (*see* USPN 5,188,958 and USPN 5,463,174).

In the first experiment, three plants were used: a control, LPO04-1, and
15 two transgenics,, 5525-23 and 5525-29. LP004 is a low-linolenic *Brassica* variety. Leaves of each were selected for one of three treatments: water, GLA or DGLA. GLA and DGLA were purchased as sodium salts from NuChek Prep and dissolved in water at 1 mg/ml. Aliquots were capped under N₂ and stored at -70 degrees C. Leaves were treated by applying a 50 μ l drop to the upper
20 surface and gently spreading with a gloved finger to cover the entire surface. Applications were made approximately 30 minutes before the end of the light cycle to minimize any photo-oxidation of the applied fatty acids. After 6 days of treatment one leaf from each treatment was harvested and cut in half through the mid rib. One half was washed with water to attempt to remove
25 unincorporated fatty acid. Leaf samples were lyophilized overnight, and fatty acid composition determined by gas chromatography (GC). The results are shown in Table 3.

Table 3
Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants

| Treatment | SPL | 16:00 | 16:01 | 18:00 | 18:01 | 18:1o | 18:1v | 18:02 | 18:3g | 18:03 | 18:04 | 20:00 | 20:01 |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | # | % | % | % | % | % | % | % | % | % | % | % | % |
| Water | 33 | 12.95 | 0.08 | 2.63 | 2.51 | 1.54 | 0.98 | 16.76 | 0 | 45.52 | 0 | 0.09 | 0 |
| | 34 | 13.00 | 0.09 | 2.67 | 2.56 | 1.55 | 1.00 | 16.86 | 0 | 44.59 | 0 | 0.15 | 0 |
| | 35 | 14.13 | 0.09 | 2.37 | 2.15 | 1.27 | 0.87 | 16.71 | 0 | 49.91 | 0 | 0.05 | 0.01 |
| | 36 | 13.92 | 0.08 | 2.32 | 2.07 | 1.21 | 0.86 | 16.16 | 0 | 50.25 | 0 | 0.05 | 0 |
| GLA | 37 | 13.79 | 0.11 | 2.10 | 2.12 | 1.26 | 0.86 | 15.90 | 0.08 | 46.29 | 0 | 0.54 | 0.01 |
| | 38 | 12.80 | 0.09 | 1.94 | 2.08 | 1.35 | 0.73 | 14.54 | 0.11 | 45.61 | 0 | 0.49 | 0.01 |
| | 39 | 12.10 | 0.09 | 2.37 | 2.10 | 1.29 | 0.82 | 14.85 | 1.63 | 43.66 | 0 | 0.53 | 0 |
| | 40 | 12.78 | 0.10 | 2.34 | 2.22 | 1.36 | 0.86 | 15.29 | 1.72 | 47.22 | 0 | 0.50 | 0.02 |
| DGLA | 41 | 13.71 | 0.07 | 2.68 | 2.16 | 1.34 | 0.82 | 15.92 | 2.12 | 46.55 | 0 | 0.09 | 0 |
| | 42 | 14.10 | 0.07 | 2.75 | 2.35 | 1.51 | 0.84 | 16.66 | 1.56 | 46.41 | 0 | 0.09 | 0.01 |
| | 43 | 13.62 | 0.09 | 2.22 | 1.94 | 1.21 | 0.73 | 14.68 | 2.42 | 46.69 | 0 | 0.51 | 0.01 |
| | 44 | 13.92 | 0.09 | 2.20 | 2.17 | 1.32 | 0.85 | 15.22 | 2.30 | 46.05 | 0 | 0.53 | 0.02 |
| | 45 | 12.45 | 0.14 | 2.30 | 2.28 | 1.37 | 0.91 | 15.65 | 0.07 | 44.62 | 0 | 0.12 | 0.01 |
| | 46 | 12.67 | 0.15 | 2.69 | 2.50 | 1.58 | 0.92 | 15.96 | 0.09 | 42.77 | 0 | 0.56 | 0.01 |
| | 47 | 12.56 | 0.23 | 3.40 | 1.98 | 1.13 | 0.86 | 13.57 | 0.03 | 45.52 | 0 | 0.51 | 0.01 |
| | 48 | 13.07 | 0.24 | 3.60 | 2.51 | 1.63 | 0.88 | 13.54 | 0.04 | 45.13 | 0 | 0.50 | 0.01 |
| | 49 | 13.26 | 0.07 | 2.81 | 2.34 | 1.67 | 0.67 | 16.04 | 0.04 | 43.89 | 0 | 0.59 | 0 |
| | 50 | 13.53 | 0.07 | 2.84 | 2.41 | 1.70 | 0.70 | 16.07 | 0.02 | 44.90 | 0 | 0.60 | 0.01 |

Table 3 - Continued
Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants

| Treatment | SPL | 20:02 | 20:03 | 20:04 | 20:05 | 22:00 | 22:01 | 22:02 | 22:03 | 22:06 | 24:0 | 24:1 |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|------|
| | # | % | % | % | % | % | % | % | % | % | % | % |
| Water | 33 | 0 | 0 | 0.29 | 0 | 0.01 | 0.09 | 16.26 | 0 | 0 | 0.38 | 0.18 |
| | 34 | 0.01 | 0 | 0.26 | 0 | 0.14 | 0.10 | 16.82 | 0.02 | 0.05 | 0.36 | 0.27 |
| | 35 | 0.01 | 0 | 0.25 | 0 | 0.12 | 0.06 | 11.29 | 0.04 | 0.05 | 0.29 | 0.25 |
| | 36 | 0 | 0.01 | 0.26 | 0 | 0.07 | 0.04 | 11.82 | 0.03 | 0.36 | 0.28 | 0.21 |
| GLA | 37 | 0.02 | 0 | 0.21 | 0 | 0.18 | 0.08 | 15.87 | 0.06 | 0.20 | 0.30 | 0.17 |
| | 38 | 0.01 | 0 | 0.24 | 0 | 0.15 | 0.07 | 13.64 | 0.09 | 0.08 | 5.89 | 0.23 |
| | 39 | 0.02 | 0.01 | 0.27 | 0 | 0.10 | 0.08 | 16.25 | 3.42 | 0.19 | 0.37 | 0.17 |
| | 40 | 0.01 | 0 | 0.27 | 0 | 0.10 | 0.10 | 14.74 | 0.05 | 0.10 | 0.36 | 0.14 |
| DGLA | 41 | 0 | 0 | 0.27 | 0 | 0.20 | 0.10 | 13.15 | 0.13 | 0.29 | 0.33 | 0.20 |
| | 42 | 0 | 0 | 0.28 | 0 | 0.11 | 0.11 | 12.60 | 0.02 | 0.24 | 0.38 | 0.13 |
| | 43 | 0.01 | 0 | 0.28 | 0 | 0.10 | 0.03 | 14.73 | 0.01 | 0.24 | 0.34 | 0.14 |
| | 44 | 0.02 | 0 | 0.26 | 0 | 0.13 | 0.07 | 14.43 | 0.05 | 0.16 | 0.33 | 0.17 |
| | 45 | 0.06 | 1.21 | 0.26 | 0 | 0.07 | 0.07 | 18.67 | 0.02 | 0.21 | 0.36 | 0.13 |
| | 46 | 0 | 1.94 | 0.27 | 0 | 0.11 | 0.09 | 17.97 | 0.09 | 0.39 | 0.41 | 0.11 |
| | 47 | 0.01 | 0.69 | 0.96 | 0 | 0.11 | 0.07 | 17.96 | 0 | 0.22 | 0.49 | 0.20 |
| | 48 | 0.01 | 0.70 | 0.74 | 0 | 0.14 | 0.09 | 17.14 | 0.05 | 0.32 | 0.52 | 0.10 |
| | 49 | 0 | 0.35 | 1.11 | 0 | 0.10 | 0.07 | 17.26 | 0.07 | 0.23 | 0.39 | 0.18 |
| | 50 | 0 | 0.20 | 0.87 | 0 | 0.21 | 0.07 | 15.73 | 0.04 | 0.15 | 0.37 | 0.18 |

Leaves treated with GLA contained from 1.56 to 2.4 wt% GLA. The fatty acid analysis showed that the lipid composition of control and transgenic leaves was essentially the same. Leaves of control plants treated with DGLA contained 1.2-1.9 w% DGLA and background amounts of ARA (.26-.27 wt%).

- 5 Transgenic leaves contained only .2-.7 wt% DGLA, but levels of ARA were increased (.74-1.1 wt%) indicating that the DGLA was converted to ARA in these leaves.

Expression in Seed

- 10 The purpose of this experiment was to determine whether a construct with the seed specific napin promoter would enable expression in seed.

The Ma29 cDNA was modified by PCR to introduce *Xho*I cloning sites upstream and downstream of the start and stop codons, respectively, using the following primers:

Madxho-forward:

- 15 5'-CUACUACUACUACTCGAGCAAGATGGGAACGGACCAAGG
(SEQ ID NO:25)

Madxho-reverse:

5'-CAUCAUCAUCAUCTCGAGCTACTCTTCCTTGGGACGGAG
(SEQ ID NO:26).

- 20 The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5522 and the $\Delta 5$ desaturase sequence was verified by sequencing of both strands.

- 25 For seed-specific expression, the Ma29 coding region was cut out of pCGN5522 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5528. The *Hind*III fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the *Hind*III site of pCGN1557 to create pCGN5531. Two copies of the napin transcriptional unit were inserted in tandem. This tandem construct can permit higher expression of

the desaturases per genetic loci. pCGN5531 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

The fatty acid composition of twenty-seed pools of mature T2 seeds was analyzed by GC. Table 4 shows the results obtained with independent
5 transformed lines as compared to non-transformed LP004 seed. The transgenic seeds containing pCGN5531 contain two fatty acids that are not present in the control seeds, tentatively identified as taxoleic acid (5,9-18:2) and pinolenic acid (5,9,12-18:3), based on their elution relative to oleic and linoleic acid. These would be the expected products of $\Delta 5$ desaturation of oleic and linoleic
10 acids. No other differences in fatty acid composition were observed in the transgenic seeds.

Table 4
Composition of T2 Pooled Seed

| | 16:0 | 16:1 | 18:0 | 18:1 | (5,9)18:2 | 18:2 | (5,9,12)18:3 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 | 22:1 | 24:0 |
|---------------|------|------|------|-------|-----------|-------|--------------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| LP004 control | 3.86 | 0.15 | 3.05 | 69.1 | 0 | 18.51 | 0.01 | 1.65 | 1.09 | 1.40 | 0.03 | 0.63 | 0.05 | 0.42 |
| 5531-1 | 4.26 | 0.15 | 3.23 | 62.33 | 4.07 | 21.44 | 0.33 | 1.38 | 0.91 | 1.04 | 0.05 | 0.41 | 0.03 | 0.27 |
| 5531-2 | 3.78 | 0.14 | 3.37 | 66.18 | 4.57 | 17.31 | 0.27 | 1.30 | 1.03 | 1.18 | 0 | 0.47 | 0.01 | 0.30 |
| 5531-6 | 3.78 | 0.13 | 3.47 | 63.61 | 6.21 | 17.97 | 0.38 | 1.34 | 1.04 | 1.14 | 0.05 | 0.49 | 0.02 | 0.26 |
| 5531-10 | 3.96 | 0.17 | 3.28 | 63.82 | 5.41 | 18.58 | 0.32 | 1.43 | 0.98 | 1.11 | 0.02 | 0.50 | 0 | 0.31 |
| 5531-16 | 3.91 | 0.17 | 3.33 | 64.31 | 5.03 | 18.98 | 0.33 | 1.39 | 0.96 | 1.11 | 0 | 0.44 | 0 | 0 |
| 5531-28 | 3.81 | 0.13 | 2.58 | 62.64 | 5.36 | 20.95 | 0.45 | 1.39 | 0.83 | 1.15 | 0.01 | 0.36 | 0.05 | 0.21 |
| | | | | | | | | | | | | | | |

Northern analysis is performed on plants to identify those expressing Ma29. Developing embryos are isolated approximately 25 days post anthesis or when the napin promoter is induced, and floated in a solution containing GLA or DGLA as described in Example 7. Fatty acid analysis of the embryos is then performed by GC to determine the amount of conversion of DGLA to ARA, following the protocol adapted for leaves in Example 7. The amount of ARA incorporated into triglycerides by endogenous *Brassica* acyltransferases is then evaluated by GC analysis as in Example 7.

Example 8

Expression of *M. alpina* $\Delta 6$ Desaturase in *Brassica napus*

The Ma524 cDNA was modified by PCR to introduce cloning sites using the following primers:

Ma524PCR-1 (SEQ ID NO:27)

5'-CUACUACUACUATCTAGACTCGAGACCATGGCTGCTGCT
CCAGTGTG

Ma524PCR-2 (SEQ ID NO:28)

5'-CAUCAUCAUCAUAGGCCTCGAGTTACTGCGCCTTACCCAT

These primers allowed the amplification of the entire coding region and added *Xba*I and *Xho*I sites to the 5'-end and *Xho*I and *Stu*I sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5535 and the $\Delta 6$ desaturase sequence was verified by sequencing of both strands.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5536. The *Not*I fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the *Not*I site of pCGN1557

to create pCGN5538. pCGN5538 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

5 Maturing T2 seeds were collected from 6 independent transformation events in the greenhouse. The fatty acid composition of single seeds was analyzed by GC. Table 5 shows the results of control LP004 seeds and six 5538 lines. All of the 5538 lines except #8 produced seeds containing GLA. Presence of GLA segregated in these seeds as is expected for the T2 selfed seed population. In addition to GLA, the *M. alpina* $\Delta 6$ desaturase is capable of producing 18:4 (stearidonic) and another fatty acid believed to be the 6,9-18:2.

10 The above results show that desaturases with three different substrate specificities can be expressed in a heterologous system and used to produce poly-unsaturated long chain fatty acids. Exemplified were the production of ARA (20:4) from the precursor 20:3 (DGLA), the production of GLA (18:3) from 18:2 substrate, and the conversion of 18:1 substrate to 18:2, which is the precursor for GLA.

15

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|-------|--------|------|------|------|------|------|------|------|
| % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| LPO04-1 | 4.33 | 0.21 | 3.78 | 72.49 | 0 | 13.97 | 0 | 1.7 | 0 | 1.34 | 0.71 | 0.02 | 0.58 | 0.27 | |
| -2 | 4.01 | 0.16 | 3.09 | 73.59 | 0 | 14.36 | 0.01 | 1.4 | 0 | 1.43 | 0.66 | 0.02 | 0.5 | 0.2 | |
| -3 | 4.12 | 0.19 | 3.56 | 70.25 | 0 | 17.28 | 0 | 1.57 | 0 | 1.28 | 0.5 | 0.02 | 0.39 | 0.2 | |
| -4 | 4.22 | 0.2 | 2.7 | 70.25 | 0 | 17.86 | 0 | 1.61 | 0 | 1.31 | 0.53 | 0.02 | 0.4 | 0.24 | |
| -5 | 4.02 | 0.16 | 3.41 | 72.91 | 0 | 14.45 | 0.01 | 1.45 | 0 | 1.37 | 0.7 | 0.02 | 0.51 | 0.26 | |
| -6 | 4.22 | 0.18 | 3.23 | 71.47 | 0 | 15.92 | 0.01 | 1.52 | 0 | 1.32 | 0.69 | 0.02 | 0.51 | 0.27 | |
| -7 | 4.1 | 0.16 | 3.47 | 72.06 | 0 | 15.23 | 0 | 1.52 | 0 | 1.32 | 0.63 | 0.03 | 0.49 | 0.23 | |
| -9 | 4.01 | 0.17 | 3.71 | 72.98 | 0 | 13.97 | 0.01 | 1.41 | 0 | 1.45 | 0.74 | 0.03 | 0.58 | 0.23 | |
| -10 | 4.04 | 0.16 | 3.57 | 70.03 | 0 | 17.46 | 0 | 1.5 | 0 | 1.33 | 0.61 | 0.03 | 0.36 | 0.24 | |
| 5538-1-1 | 4.61 | 0.2 | 3.48 | 68.12 | 1.37 | 10.68 | 7.48 | 1.04 | 0.33 | 1.19 | 0.49 | 0.02 | 0.33 | 0.13 | |
| -2 | 4.61 | 0.22 | 3.46 | 68.84 | 1.36 | 10.28 | 7.04 | 1.01 | 0.31 | 1.15 | 0.48 | 0.02 | 0.39 | 0 | |
| -3 | 4.78 | 0.24 | 3.24 | 65.86 | 0 | 21.36 | 0 | 1.49 | 0 | 1.08 | 0.46 | 0.02 | 0.38 | 0.22 | |
| -4 | 4.84 | 0.3 | 3.89 | 67.64 | 1.67 | 9.9 | 6.97 | 1.02 | 0.36 | 1.14 | 0.53 | 0.02 | 0.5 | 0.18 | |
| -5 | 4.64 | 0.2 | 3.58 | 64.5 | 3.61 | 8.85 | 10.14 | 0.95 | 0.48 | 1.19 | 0.47 | 0.01 | 0.33 | 0.12 | |
| -6 | 4.91 | 0.27 | 3.44 | 66.51 | 1.48 | 11.14 | 7.74 | 1.15 | 0.33 | 1.08 | 0.49 | 0.02 | 0.34 | 0.13 | |
| -7 | 4.87 | 0.22 | 3.24 | 65.78 | 1.27 | 11.92 | 8.38 | 1.2 | 0 | 1.12 | 0.47 | 0.02 | 0.37 | 0.16 | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|------|------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -8 | 4.59 | 0.22 | 3.4 | 70.77 | 0 | 16.71 | 0 | 1.35 | 0 | 1.14 | 0.48 | 0.02 | 0.39 | 0.15 | | |
| -9 | 4.63 | 0.23 | 3.51 | 69.66 | 2.01 | 8.77 | 7.24 | 0.97 | 0 | 1.18 | 0.52 | 0.02 | 0.3 | 0.11 | | |
| -10 | 4.56 | 0.19 | 3.55 | 70.68 | 0 | 16.89 | 0 | 1.37 | 0 | 1.22 | 0.54 | 0.02 | 0.22 | 0.03 | | |
| 5538-3-1 | 4.74 | 0.21 | 3.43 | 67.52 | 1.29 | 10.91 | 7.77 | 1.03 | 0.28 | 1.11 | 0.5 | 0.02 | 0.35 | 0.14 | | |
| -2 | 4.72 | 0.21 | 3.24 | 67.42 | 1.63 | 10.37 | 8.4 | 0.99 | 0 | 1.12 | 0.49 | 0.02 | 0.36 | 0.15 | | |
| -3 | 4.24 | 0.21 | 3.52 | 71.31 | 0 | 16.53 | 0 | 1.33 | 0 | 1.12 | 0.45 | 0.02 | 0.4 | 0.14 | | |
| -4 | 4.64 | 0.21 | 3.45 | 67.92 | 1.65 | 9.91 | 7.97 | 0.91 | 0.33 | 1.14 | 0.47 | 0.02 | 0.37 | 0.14 | | |
| -5 | 4.91 | 0.25 | 3.31 | 67.19 | 0 | 19.92 | 0.01 | 1.39 | 0 | 1.05 | 0.48 | 0.02 | 0.37 | 0.14 | | |
| -6 | 4.67 | 0.21 | 3.25 | 67.07 | 1.23 | 11.32 | 8.35 | 0.99 | 0 | 1.16 | 0.47 | 0.02 | 0.33 | 0.16 | | |
| -7 | 4.53 | 0.19 | 2.94 | 64.8 | 4.94 | 8.45 | 9.95 | 0.93 | 0.44 | 1.13 | 0.37 | 0.01 | 0.27 | 0.12 | | |
| -8 | 4.66 | 0.22 | 3.68 | 67.33 | 0.71 | 12 | 6.99 | 1.1 | 0.24 | 1.18 | 0.48 | 0.03 | 0.36 | 0.17 | | |
| -9 | 4.65 | 0.24 | 3.11 | 67.42 | 0.64 | 12.71 | 6.93 | 1.16 | 0.25 | 1.08 | 0.45 | 0.02 | 0.32 | 0.17 | | |
| -10 | 4.88 | 0.27 | 3.33 | 65.75 | 0.86 | 12.89 | 7.7 | 1.1 | 0.24 | 1.08 | 0.46 | 0.01 | 0.34 | 0.16 | | |
| 5538-4-1 | 4.65 | 0.24 | 3.8 | 62.41 | 0 | 24.68 | 0 | 1.6 | 0.01 | 0.99 | 0.45 | 0.02 | 0.33 | 0.13 | | |
| -2 | 5.37 | 0.31 | 3 | 57.98 | 0.38 | 18.04 | 10.5 | 1.41 | 0 | 0.99 | 0.48 | 0.02 | 0.3 | 0.19 | | |
| -3 | 4.61 | 0.22 | 3.07 | 63.62 | 0.3 | 16.46 | 7.67 | 1.2 | 0 | 1.18 | 0.45 | 0.02 | 0.29 | 0.14 | | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|-------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -4 | 4.39 | 0.19 | 2.93 | 65.97 | 0 | 22.36 | 0 | 1.45 | 0 | 1.17 | 0.41 | 0.03 | 0.32 | 0.15 | |
| -5 | 5.22 | 0.29 | 3.85 | 62.1 | 2.35 | 10.25 | 11.39 | 0.93 | 0.41 | 1.04 | 0.6 | 0.02 | 0.47 | 0.17 | |
| -6 | 4.66 | 0.18 | 2.85 | 66.79 | 0.5 | 13.03 | 7.66 | 0.97 | 0.22 | 1.28 | 0.42 | 0.02 | 0.31 | 0.14 | |
| -7 | 4.85 | 0.26 | 3.03 | 57.43 | 0.26 | 28.04 | 0.01 | 2.59 | 0.01 | 1.13 | 0.56 | 0.02 | 0.4 | 0.23 | |
| -8 | 5.43 | 0.28 | 2.94 | 54.8 | 1.84 | 13.79 | 15.67 | 1.36 | 0.53 | 1.1 | 0.55 | 0.02 | 0.35 | 0.19 | |
| -9 | 4.88 | 0.24 | 3.32 | 62.3 | 0.58 | 14.86 | 9.04 | 1.34 | 0.29 | 1.13 | 0.52 | 0.02 | 0.37 | 0.19 | |
| -10 | 4.53 | 0.2 | 2.73 | 64.2 | 0.07 | 24.15 | 0 | 1.52 | 0 | 1.09 | 0.39 | 0.02 | 0.27 | 0.17 | |
| 5538-5-1 | 4.5 | 0.15 | 3.35 | 66.71 | 0.88 | 11.7 | 8.38 | 1.04 | 0.3 | 1.24 | 0.49 | 0.02 | 0.29 | 0.17 | |
| -2 | 4.77 | 0.23 | 3.06 | 62.67 | 0.68 | 15.2 | 8.8 | 1.31 | 0.28 | 1.15 | 0.46 | 0.02 | 0.3 | 0.19 | |
| -3 | 4.59 | 0.22 | 3.61 | 64.35 | 2.29 | 9.95 | 10.57 | 1.01 | 0.45 | 1.21 | 0.48 | 0.02 | 0.26 | 0.16 | |
| -4 | 4.86 | 0.26 | 3.4 | 67.69 | 0.65 | 12.24 | 6.61 | 1.09 | 0.23 | 1.07 | 0.45 | 0.02 | 0.32 | 0.14 | |
| -5 | 4.49 | 0.21 | 3.3 | 69.25 | 0.04 | 16.51 | 2.18 | 1.2 | 0 | 1.11 | 0.44 | 0.02 | 0.33 | 0.16 | |
| -6 | 4.5 | 0.21 | 3.47 | 70.48 | 0.08 | 14.9 | 2.19 | 1.22 | 0 | 1.13 | 0.49 | 0.02 | 0.33 | 0.16 | |
| -7 | 4.39 | 0.21 | 3.44 | 67.59 | 2.38 | 9.24 | 8.98 | 0.89 | 0 | 1.18 | 0.44 | 0.02 | 0.28 | 0.14 | |
| -8 | 4.52 | 0.22 | 3.17 | 68.33 | 0.01 | 18.91 | 0.73 | 1.32 | 0.01 | 1.08 | 0.45 | 0.02 | 0.29 | 0.17 | |
| -9 | 4.68 | 0.2 | 3.05 | 64.03 | 1.93 | 11.03 | 11.41 | 1.02 | 0.01 | 1.15 | 0.39 | 0.02 | 0.21 | 0.15 | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|-----------|------|------|------|-------|------|-------|------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -10 | 4.57 | 0.2 | 3.1 | 67.21 | 0.61 | 12.62 | 7.68 | 1.07 | 0.25 | 1.14 | 0.43 | 0.02 | 0.25 | 0.15 | |
| 5538-8-1 | 4.95 | 0.26 | 3.14 | 64.04 | 0 | 23.38 | 0 | 1.54 | 0 | 0.99 | 0.42 | 0.02 | 0.38 | 0.17 | |
| -2 | 4.91 | 0.26 | 3.71 | 62.33 | 0 | 23.97 | 0 | 1.77 | 0 | 0.95 | 0.53 | 0.02 | 0.42 | 0.19 | |
| -3 | 4.73 | 0.25 | 4.04 | 63.83 | 0 | 22.36 | 0.01 | 1.73 | 0 | 1.05 | 0.55 | 0.02 | 0.45 | 0.16 | |
| -4 | 5.1 | 0.35 | 3.8 | 60.45 | 0 | 24.45 | 0.01 | 2.13 | 0 | 1.07 | 0.65 | 0.03 | 0.53 | 0.24 | |
| -5 | 4.98 | 0.3 | 3.91 | 62.48 | 0 | 23.44 | 0 | 1.77 | 0 | 1.01 | 0.51 | 0.01 | 0.43 | 0.21 | |
| -6 | 4.62 | 0.21 | 3.99 | 66.14 | 0 | 20.38 | 0 | 1.48 | 0 | 1.15 | 0.53 | 0.02 | 0.48 | 0.19 | |
| -7 | 4.64 | 0.22 | 3.55 | 64.6 | 0 | 22.65 | 0 | 1.38 | 0 | 1.09 | 0.45 | 0.02 | 0.41 | 0.19 | |
| -8 | 5.65 | 0.38 | 3.18 | 56.6 | 0 | 30.83 | 0.02 | 0.02 | 0 | 0.98 | 0.55 | 0.03 | 0.39 | 0.26 | |
| -9 | 8.53 | 0.63 | 6.9 | 51.76 | 0 | 26.01 | 0 | 0.01 | 0 | 1.41 | 1.21 | 0.07 | 0.96 | 0.33 | |
| -10 | 5.52 | 0.4 | 3.97 | 57.92 | 0 | 28.95 | 0 | 0.02 | 0 | 0.95 | 0.52 | 0.02 | 0.41 | 0.16 | |
| 5538-10-1 | 4.44 | 0.19 | 3.5 | 68.42 | 0 | 19.51 | 0 | 1.32 | 0 | 1.14 | 0.45 | 0.02 | 0.31 | 0.16 | |
| -2 | 4.57 | 0.21 | 3.07 | 66.08 | 0 | 21.99 | 0.01 | 1.36 | 0 | 1.12 | 0.41 | 0.02 | 0.31 | 0.16 | |
| -3 | 4.63 | 0.21 | 3.48 | 67.43 | 0 | 20.27 | 0.01 | 1.32 | 0 | 1.12 | 0.46 | 0.02 | 0.21 | 0.08 | |
| -4 | 4.69 | 0.19 | 3.22 | 64.62 | 0 | 23.16 | 0 | 1.35 | 0 | 1.08 | 0.46 | 0.02 | 0.33 | 0.2 | |
| -5 | 4.58 | 0.2 | 3.4 | 68.75 | 0 | 20.17 | 0.01 | 0.02 | 0 | 1.1 | 0.45 | 0.02 | 0.34 | 0.17 | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL | # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|-----|-----|------|------|------|-------|------|-------|------|--------|------|------|------|------|------|------|------|
| | | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| | -8 | 4.55 | 0.21 | 0 | 73.55 | 0.05 | 14.91 | 2.76 | 1.21 | 0.07 | 1.24 | 0.51 | 0.02 | 0.19 | 0 | |
| | -9 | 4.58 | 0.21 | 3.28 | 66.19 | 0 | 21.55 | 0 | 1.35 | 0 | 1.12 | 0.43 | 0.02 | 0.33 | 0.16 | |
| | -10 | 4.52 | 0.2 | 3.4 | 68.37 | 0 | 19.33 | 0.01 | 1.3 | 0 | 1.13 | 0.46 | 0.02 | 0.35 | 0.18 | |

Example 9**Expression of *M. alpina* $\Delta 12$ desaturase in *Brassica napus***

The Ma648 cDNA was modified by PCR to introduce cloning sites using the following primers:

5 Ma648PCR-for (SEQ ID NO:29)
 5'-CUACUACUACUAGGATCCATGGCACCTCCCAACACT
 Ma648PCR-rev (SEQ ID NO:30)
 5'-CAUCAUCAUCAUGGTACCTCGAGTTACTTCTTGAAAAAGAC

10 These primers allowed the amplification of the entire coding region and added a BamHI site to the 5' end and KpnI and XhoI sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5540 and the $\Delta 12$ desaturase sequence was verified by sequencing of both strands.

15 For seed-specific expression, the Ma648 coding region was cut out of pCGN5540 as a BamHI/XhoI fragment and inserted between the BglII and XhoI sites of the napin expression cassette, pCGN3223, to create pCGN5542. The Asp718 fragment of pCGN5541 containing the napin 5' regulatory region, the Ma648 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5542. PCGN5542 was
 20 introduced into two varieties of *Brassica napus* via *Agrobacterium* mediated transformation. The commercial canola variety, SP30021, and a low-linolenic line, LP30108 were used.

25 Mature selfed T2 seeds were collected from 19 independent LP30108 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 6. All transformed lines contained increased levels of 18:2, the product of the $\Delta 12$ desaturase. Levels of 18:3 were not significantly increased in these plants. Events # 11 and 16 showed the greatest accumulation

of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 7. Individual T2 seeds containing the *M. alpina* $\Delta 12$ desaturase accumulated up to 60% 18:2 in the seeds. Sample 97xx1116 #59 is an example of a null segregant. Even in the highest 18:2 accumulators, levels of 18:3 were increased only slightly. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed.

Mature selfed T2 seeds were collected from 20 independent SP30021 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The data are presented in Table 8. All transformed lines contained increased levels of 18:2, the product of the $\Delta 12$ desaturase. As in the low-linolenic LP30108 line, levels of 18:3 were not significantly increased. Events # 4 and 12 showed the greatest accumulation of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 9. Samples 97xx1157 #88 and #18 are examples of null segregants for 5542-SP30021-4 and 5542-SP30021-12 respectively. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed

Table 6

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| 97XX1098 | 45 | 5542-LP30108-16 | 7.04 | 0.43 | 1.12 | 18.01 | 66.36 | 4.76 | 0.5 | 0.84 | 0.3 | 0.44 |
| 97XX1098 | 22 | 5542-LP30108-16 | 5.17 | 0.29 | 2.11 | 22.01 | 65.18 | 3.15 | 0.63 | 0.75 | 0.21 | 0.36 |
| 97XX1098 | 40 | 5542-LP30108-16 | 4.99 | 0.2 | 2.05 | 23.91 | 63.13 | 3.3 | 0.73 | 0.85 | 0.23 | 0.49 |
| 97XX1098 | 28 | 5542-LP30108-16 | 4.47 | 0.19 | 1.75 | 26.7 | 62.39 | 2.46 | 0.58 | 0.85 | 0.2 | 0.32 |
| 97XX1098 | 2 | 5542-LP30108-16 | 4.54 | 0.21 | 1.66 | 26.83 | 61.89 | 2.9 | 0.55 | 0.82 | 0.18 | 0.33 |
| 97XX1098 | 58 | 5542-LP30108-16 | 6.05 | 0.31 | 1.36 | 24.11 | 61.36 | 3.8 | 0.72 | 1.13 | 0.26 | 0.58 |
| 97XX1098 | 83 | 5542-LP30108-16 | 5.13 | 0.17 | 2.03 | 27.05 | 60.93 | 2.62 | 0.7 | 0.71 | 0.14 | 0.4 |
| 97XX1098 | 34 | 5542-LP30108-16 | 4.12 | 0.19 | 1.44 | 29.35 | 60.54 | 2.53 | 0.43 | 0.89 | 0.17 | 0.25 |
| 97XX1116 | 37 | 5542-LP30108-11 | 4 | 0.14 | 2.43 | 23.29 | 63.99 | 2.6 | 0.58 | 0.69 | 0.71 | 1.11 |
| 97XX1116 | 88 | 5542-LP30108-11 | 3.8 | 0.18 | 2.04 | 23.59 | 63.93 | 2.95 | 0.54 | 0.81 | 0.99 | 0.82 |
| 97XX1116 | 36 | 5542-LP30108-11 | 4.15 | 0.2 | 1.51 | 25.94 | 62.14 | 2.74 | 0.47 | 0.87 | 0.79 | 0.81 |
| 97XX1116 | 31 | 5542-LP30108-11 | 6.29 | 0.35 | 1.04 | 24.14 | 60.91 | 4.02 | 0.55 | 0.91 | 0.75 | 0.72 |
| 97XX1116 | 10 | 5542-LP30108-11 | 6.97 | 0.4 | 3.36 | 18.9 | 60.66 | 4.68 | 1.2 | 0.7 | 0.53 | 1.71 |
| 97XX1116 | 32 | 5542-LP30108-11 | 3.96 | 0.16 | 2.61 | 26.73 | 60.54 | 3.38 | 0.66 | 0.87 | 0.2 | 0.62 |
| 97XX1116 | 55 | 5542-LP30108-11 | 4.26 | 0.22 | 0.98 | 28.57 | 59.94 | 3.24 | 0.4 | 0.68 | 0.71 | 0.75 |
| 97XX1116 | 12 | 5542-LP30108-11 | 4.17 | 0.23 | 1.42 | 28.61 | 59.52 | 3.26 | 0.51 | 0.95 | 0.29 | 0.67 |

Table 6

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| 97XX1116 | 86 | 5542-LP30108-11 | 4.23 | 0.3 | 1.09 | 28.34 | 59.2 | 3.95 | 0.48 | 0.91 | 0.55 | 0.71 |
| 97XX1116 | 61 | 5542-LP30108-11 | 4.13 | 0.16 | 1.92 | 30.18 | 58.67 | 2.65 | 0.56 | 0.88 | 0.25 | 0.41 |
| 97XX1116 | 60 | 5542-LP30108-11 | 4.42 | 0.26 | 1.61 | 28.77 | 58.6 | 3.26 | 0.53 | 0.85 | 0.68 | 0.75 |
| 97XX1116 | 91 | 5542-LP30108-11 | 7.82 | 0.67 | 2.37 | 17.97 | 58.43 | 4.85 | 0.94 | 0.86 | 3.87 | 1.71 |
| 97xx1116 | 59 | 5542-LP30108-11 | 3.56 | 0.2 | 1.6 | 65.5 | 23.03 | 2.23 | 0.52 | 1.54 | 0.49 | 0.69 |

Table 7

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % |
| 5542-LP30108-1 | 4.6 | 0.15 | 1.93 | 50.44 | 38.54 | 2.06 | 0.65 | 1.11 | 0.09 | 0.37 |
| 5542-LP30108-2 | 4.63 | 0.17 | 1.78 | 41.11 | 47.53 | 2.46 | 0.62 | 1.02 | 0.14 | 0.38 |
| 5542-LP30108-3 | 4.96 | 0.18 | 2.07 | 48.16 | 40.01 | 2.17 | 0.73 | 1.13 | 0.1 | 0.39 |
| 5542-LP30108-4 | 4.36 | 0.15 | 1.94 | 46.51 | 42.57 | 1.95 | 0.64 | 1.06 | 0.11 | 0.35 |
| 5542-LP30108-5 | 4.45 | 0.14 | 2.19 | 49.54 | 39.13 | 2.14 | 0.72 | 1.14 | 0.11 | 0.38 |
| 5542-LP30108-6 | 4.97 | 0.16 | 1.86 | 49.23 | 39.2 | 2.17 | 0.7 | 1.12 | 0.11 | 0.41 |
| 5542-LP30108-7 | 4.46 | 0.13 | 2.72 | 39.6 | 48.65 | 2.02 | 0.81 | 0.96 | 0.13 | 0.4 |
| 5542-LP30108-8 | 4.63 | 0.18 | 1.78 | 47.86 | 41 | 2.31 | 0.62 | 1.09 | 0.11 | 0.36 |
| 5542-LP30108-9 | 4.64 | 0.16 | 1.75 | 42.5 | 46.57 | 2.2 | 0.61 | 1 | 0.13 | 0.35 |
| 5542-LP30108-10 | 4.46 | 0.15 | 2.37 | 43.61 | 45.29 | 1.77 | 0.71 | 1.02 | 0.12 | 0.36 |
| 5542-LP30108-11 | 4.58 | 0.25 | 1.88 | 37.08 | 50.95 | 2.94 | 0.64 | 0.96 | 0.16 | 0.42 |
| 5542-LP30108-12 | 4.46 | 0.18 | 1.69 | 43.62 | 45.36 | 2.44 | 0.59 | 1.09 | 0.14 | 0.34 |
| 5542-LP30108-13 | 4.45 | 0.15 | 2.33 | 51 | 37.71 | 1.91 | 0.75 | 1.12 | 0.09 | 0.4 |
| 5542-LP30108-14 | 4.3 | 0.16 | 2.04 | 45.93 | 42.78 | 2.46 | 0.66 | 1.07 | 0.14 | 0.37 |
| 5542-LP30108-15 | 4.18 | 0.16 | 2.17 | 43.79 | 45.2 | 2.14 | 0.68 | 1.04 | 0.15 | 0.36 |
| 5542-LP30108-16 | 5.04 | 0.18 | 1.89 | 32.32 | 55.78 | 2.68 | 0.63 | 0.84 | 0.2 | 0.36 |

Table 7

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % |
| 5542-LP30108-18 | 4.2 | 0.14 | 2.23 | 50.63 | 38.51 | 1.79 | 0.72 | 1.15 | 0.1 | 0.37 |
| 5542-LP30108-19 | 4.63 | 0.18 | 1.81 | 52.51 | 36.26 | 2.12 | 0.68 | 1.19 | 0.1 | 0.4 |
| 5542-LP30108-20 | 4.77 | 0.15 | 2.78 | 39.76 | 48.06 | 2.25 | 0.75 | 0.91 | 0.13 | 0.36 |
| LP30108 control | 4.31 | 0.22 | 2.05 | 66.15 | 22.59 | 1.87 | 0.77 | 1.3 | 0.07 | 0.44 |

Table 8

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 5542-SP30021-1 | 4.37 | 0.17 | 2.17 | 40.26 | 39.43 | 11.06 | 0.74 | 1.14 | 0.14 | 0.42 |
| 5542-SP30021-2 | 4.33 | 0.18 | 1.51 | 43.07 | 36.03 | 12.57 | 0.57 | 1.21 | 0.14 | 0.33 |
| 5542-SP30021-3 | 5.2 | 0.22 | 3.1 | 43.7 | 37.04 | 8.03 | 0.92 | 1.06 | 0.13 | 0.48 |
| 5542-SP30021-4 | 4.37 | 0.15 | 1.94 | 34.26 | 45.12 | 12.04 | 0.6 | 0.96 | 0.17 | 0.3 |
| 5542-SP30021-5 | 4.15 | 0.17 | 1.73 | 48.98 | 31.13 | 11.41 | 0.63 | 1.26 | 0.13 | 0.35 |
| 5542-SP30021-6 | 4.52 | 0.17 | 1.92 | 38.1 | 42.39 | 10.53 | 0.67 | 1.04 | 0.18 | 0.39 |
| 5542-SP30021-7 | 4.58 | 0.18 | 1.66 | 41.87 | 37.52 | 11.8 | 0.62 | 1.14 | 0.15 | 0.36 |
| 5542-SP30021-8 | 4.46 | 0.17 | 1.59 | 42.69 | 36.93 | 11.88 | 0.59 | 1.14 | 0.14 | 0.35 |
| 5542-SP30021-9 | 4.63 | 0.19 | 1.69 | 39.89 | 39.75 | 11.48 | 0.62 | 1.09 | 0.15 | 0.38 |
| 5542-SP30021-10 | 4.74 | 0.16 | 1.79 | 39.19 | 40.51 | 11.42 | 0.63 | 0.99 | 0.13 | 0.34 |
| 5542-SP30021-11 | 4.57 | 0.16 | 1.71 | 38.13 | 42 | 11.15 | 0.62 | 1.04 | 0.18 | 0.36 |
| 5542-SP30021-12 | 4.05 | 0.16 | 2.04 | 35.44 | 43.47 | 12.45 | 0.62 | 1.07 | 0.21 | 0.33 |
| 5542-SP30021-13 | 4.37 | 0.15 | 1.79 | 38.74 | 41.28 | 11.36 | 0.62 | 1.04 | 0.16 | 0.35 |
| 5542-SP30021-14 | 4.32 | 0.16 | 1.47 | 42.32 | 37.17 | 12.3 | 0.54 | 1.16 | 0.16 | 0.32 |
| 5542-SP30021-15 | 4.25 | 0.18 | 1.65 | 44.96 | 34.28 | 12.39 | 0.59 | 1.13 | 0.14 | 0.32 |

Table 8

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 5542-SP30021-16 | 4.53 | 0.17 | 1.91 | 42.13 | 38.32 | 10.51 | 0.67 | 1.12 | 0.14 | 0.38 |
| 5542-SP30021-17 | 4.16 | 0.19 | 1.7 | 50.65 | 29.3 | 11.4 | 0.61 | 1.29 | 0.11 | 0.36 |
| 5542-SP30021-18 | 4.24 | 0.17 | 1.68 | 44.47 | 35.46 | 11.52 | 0.6 | 1.19 | 0.14 | 0.34 |
| 5542-SP30021-19 | 4.1 | 0.18 | 1.8 | 46.67 | 33.87 | 10.86 | 0.63 | 1.24 | 0.13 | 0.37 |
| 5542-SP30021-20 | 4.3 | 0.17 | 1.64 | 39.6 | 40.39 | 11.53 | 0.57 | 1.12 | 0.16 | 0.32 |
| SP30021 | 4.38 | 0.21 | 1.47 | 56.51 | 22.59 | 12.04 | 0.62 | 1.45 | 0.11 | 0.39 |

Table 9

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 97XX1156 | 96 | 5542-SP30021-4 | 3.71 | 0.13 | 1.36 | 29.29 | 51.74 | 11.57 | 0.41 | 0.85 | 0.18 | 0.46 |
| 97XX1156 | 50 | 5542-SP30021-4 | 2.95 | 0.11 | 1.33 | 28.78 | 50.97 | 13.83 | 0.3 | 0.99 | 0.28 | 0.32 |
| 97XX1158 | 10 | 5542-SP30021-4 | 4.05 | 0.16 | 2.47 | 31.18 | 50.88 | 8.77 | 0.67 | 0.89 | 0.22 | 0.33 |
| 97XX1158 | 32 | 5542-SP30021-4 | 3.56 | 0.15 | 1.44 | 30.73 | 50.1 | 11.86 | 0.47 | 0.91 | 0.21 | 0.22 |
| 97XX1158 | 56 | 5542-SP30021-4 | 4.44 | 0.19 | 3.09 | 30.64 | 49.71 | 9.39 | 0.83 | 0.79 | 0.2 | 0.4 |
| 97XX1157 | 80 | 5542-SP30021-4 | 4.05 | 0.18 | 1.32 | 27.41 | 49.59 | 14.81 | 0.53 | 1.19 | 0.29 | 0.4 |
| 97XX1158 | 39 | 5542-SP30021-4 | 4.04 | 0.15 | 2.98 | 28.62 | 49.52 | 12.28 | 0.69 | 0.86 | 0.31 | 0.27 |
| 97XX1156 | 17 | 5542-SP30021-4 | 3.65 | 0.15 | 2.43 | 29.38 | 49.42 | 12.3 | 0.52 | 0.92 | 0.67 | 0.35 |
| 97XX1156 | 60 | 5542-SP30021-4 | 3.75 | 0.17 | 1.7 | 30.03 | 49.13 | 12.87 | 0.51 | 1.01 | 0.27 | 0.35 |
| 97XX1157 | 83 | 5542-SP30021-4 | 4.15 | 0.2 | 1.77 | 29.72 | 49.08 | 12.22 | 0.66 | 1.21 | 0.16 | 0.52 |
| 97XX1157 | 86 | 5542-SP30021-4 | 3.6 | 0.14 | 1.12 | 27.65 | 49.01 | 16.05 | 0.48 | 1.21 | 0.33 | 0.08 |
| 97XX1158 | 77 | 5542-SP30021-4 | 4.14 | 0.17 | 1.58 | 31.98 | 48.82 | 10.72 | 0.65 | 1 | 0.28 | 0.44 |
| 97XX1157 | 88 | 5542-SP30021-4 | 3.36 | 0.15 | 1.22 | 56.42 | 21.63 | 13.78 | 0.58 | 1.85 | 0.06 | 0.65 |

Table 9

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 97XX1157 | 39 | 5542-SP30021-12 | 2.84 | 0.04 | 1.84 | 29.6 | 53.16 | 9.52 | 0.57 | 1.32 | 0.35 | 0.48 |
| 97XX1157 | 55 | 5542-SP30021-12 | 3.28 | 0.1 | 2.18 | 30.36 | 52.27 | 9.26 | 0.63 | 1.15 | 0.22 | 0.41 |
| 97XX1157 | 10 | 5542-SP30021-12 | 3.5 | 0.06 | 1.51 | 29.78 | 50.98 | 11.13 | 0.64 | 1.45 | 0.4 | 0.26 |
| 97XX1157 | 41 | 5542-SP30021-12 | 3.31 | 0.08 | 1.64 | 30.18 | 50.51 | 11.59 | 0.57 | 1.27 | 0.24 | 0.41 |
| 97XX1157 | 35 | 5542-SP30021-12 | 3.31 | 0.09 | 1.57 | 30.36 | 50.1 | 12.17 | 0.5 | 1.15 | 0.23 | 0.35 |
| 97XX1157 | 1 | 5542-SP30021-12 | 3.45 | 0.11 | 2.88 | 32.11 | 49.45 | 8.69 | 0.82 | 1.22 | 0.27 | 0.63 |
| 97XX1157 | 16 | 5542-SP30021-12 | 2.91 | 0.09 | 1.52 | 29.35 | 48.88 | 14.26 | 0.58 | 1.39 | 0.15 | 0.3 |
| 97XX1157 | 50 | 5542-SP30021-12 | 3.29 | 0.09 | 2.13 | 33.23 | 48.78 | 9.87 | 0.67 | 1.06 | 0.18 | 0.47 |
| 97XX1157 | 25 | 5542-SP30021-12 | 2.83 | 0.05 | 1.4 | 33.22 | 48.52 | 11.22 | 0.5 | 1.33 | 0.26 | 0.42 |
| 97XX1157 | 57 | 5542-SP30021-12 | 2.94 | 0.13 | 1.46 | 32.85 | 47.58 | 12.21 | 0.57 | 1.31 | 0.27 | 0.47 |
| 97XX1157 | 56 | 5542-SP30021-12 | 3.01 | 0.07 | 1.63 | 31.53 | 47 | 14.02 | 0.59 | 1.31 | 0.28 | 0.23 |
| 97XX1157 | 6 | 5542-SP30021-12 | 3.9 | 0.13 | 1.5 | 32.43 | 46.98 | 12.45 | 0.52 | 1.11 | 0.21 | 0.49 |
| 97XX1157 | 18 | 5542-SP30021-12 | 3.88 | 0.16 | 1.73 | 57.94 | 22.33 | 10.51 | 0.74 | 1.68 | 0.11 | 0.64 |

Example 10

Simultaneous expression of *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5 In order to express the *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases from the same T-DNA, the following construct for seed-specific expression was made.

The NotI fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5542 to create pCGN5544. The
10 expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma648 and the nptII marker is the same.

PCGN5544 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 16 independent LP30108 transformation events and a non-transformed
15 control that were grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 6 + \Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are presented in Table 10. All but one of the lines (5544-LP30108-3) shows an altered oil composition as compared to the controls. GLA was produced in all but three of the lines (-3, -4,
20 -11); two of the three without GLA (-4, -11) showed increased 18:2 indicative of expression of the $\Delta 12$ desaturase. As a group, the levels of GLA observed in plants containing the double $\Delta 6 + \Delta 12$ construct (pCGN5544) were higher than those of plants containing pCGN5538 ($\Delta 6$ alone). In addition, levels of the $\Delta^{6,9}$ 18:2 are much reduced in the plants containing the $\Delta 12 + \Delta 6$ as compared to $\Delta 6$
25 alone. Thus, the combination of $\Delta 6$ and $\Delta 12$ desaturases on one T-DNA leads to the accumulation of more GLA and fewer side products than expression of $\Delta 6$ desaturase alone. To investigate the segregation of GLA levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30
30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of

these analyses are shown in Table 11. As expected for the T2 population, levels of GLA and 18:2 are segregating in the individual seeds. GLA content of up to 60% of total fatty acids was observed in individual seeds. Individual events were selected to be grown in the greenhouse and field for production of T3 seed.

Transgenic plants including *Brassica*, soybean, safflower, corn flax and sunflower expressing the constructs of this invention can be a good source of GLA.

Typical sources of GLA such as borage produce at most 25% GLA. In contrast the plants in Table 10 contain up to 30% GLA. Furthermore, the individual seeds shown in Table 11 contain up to 60% GLA.

Table 10

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:2 | 18:2 | 18:3 | 18:3 | 18:4 | 20:0 | 20:1 | 22:0 |
|-----------------|------|------|------|-------|------|--------------|---------------|-----------------|------------------|------|------|------|------|
| | | | | | | $\Delta 6,9$ | $\Delta 9,12$ | $\Delta 6,9,12$ | $\Delta 9,12,15$ | | | | |
| | % | % | % | % | % | % | % | % | % | % | % | % | % |
| 5544-LP30108-1 | 4.54 | 0.17 | 1.91 | 49.96 | 0 | 30.98 | 7.97 | 1.85 | 0.11 | 0.68 | 1.17 | 0.41 | |
| 5544-LP30108-2 | 4.69 | 0.19 | 2.15 | 38.49 | 0 | 33.94 | 16.21 | 1.73 | 0.25 | 0.72 | 0.96 | 0.41 | |
| 5544-LP30108-3 | 4.26 | 0.2 | 1.97 | 66.68 | 0 | 22.13 | 0.08 | 1.96 | 0.01 | 0.73 | 1.33 | 0.42 | |
| 5544-LP30108-4 | 4.59 | 0.24 | 1.76 | 44.21 | 0 | 44.54 | 0.02 | 2.19 | 0.01 | 0.62 | 1.08 | 0.4 | |
| 5544-LP30108-5 | 4.5 | 0.18 | 2.28 | 47.57 | 0 | 26.41 | 14.42 | 1.71 | 0.22 | 0.78 | 1.1 | 0.43 | |
| 5544-LP30108-6 | 4.51 | 0.16 | 2.12 | 31.95 | 0.01 | 26.94 | 29.8 | 1.41 | 0.5 | 0.81 | 1.02 | 0.51 | |
| 5544-LP30108-7 | 4.84 | 0.21 | 1.68 | 38.24 | 0 | 32.27 | 18.21 | 1.87 | 0.33 | 0.66 | 1.04 | 0.43 | |
| 5544-LP30108-10 | 5 | 0.28 | 1.86 | 41.17 | 0 | 46.54 | 0.36 | 2.58 | 0.02 | 0.6 | 0.91 | 0.37 | |
| 5544-LP30108-11 | 4.57 | 0.2 | 1.74 | 47.29 | 0 | 41.49 | 0.03 | 2.22 | 0.01 | 0.64 | 1.17 | 0.4 | |
| 5544-LP30108-12 | 4.87 | 0.18 | 2.65 | 34.53 | 0 | 30.37 | 23.12 | 1.46 | 0.36 | 0.83 | 0.95 | 0.45 | |
| 5544-LP30108-13 | 4.41 | 0.16 | 2.32 | 40.82 | 0.11 | 26.8 | 21.05 | 1.53 | 0.37 | 0.77 | 1.06 | 0.42 | |
| 5544-LP30108-14 | 4.38 | 0.2 | 2.21 | 29.91 | 0.16 | 28.01 | 30.62 | 1.46 | 0.59 | 0.76 | 0.97 | 0.47 | |
| 5544-LP30108-15 | 4.79 | 0.22 | 2.23 | 23.42 | 0.02 | 28.73 | 35.68 | 1.51 | 0.77 | 0.87 | 0.89 | 0.56 | |
| 5544-LP30108-16 | 4.54 | 0.18 | 1.78 | 40.81 | 0 | 35.24 | 12.83 | 1.95 | 0.27 | 0.68 | 1.02 | 0.43 | |
| 5544-LP30108-17 | 4.63 | 0.18 | 2.28 | 46.96 | 0 | 31.06 | 10.6 | 1.7 | 0.14 | 0.76 | 1.06 | 0.42 | |
| 5544-LP30108-20 | 4.87 | 0.29 | 1.44 | 31.81 | 0.15 | 23.51 | 32.85 | 1.64 | 0.69 | 0.89 | 0.96 | 0.67 | |

Table 10

| 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:2 | 18:3 | 18:3 | 18:4 | 20:0 | 20:1 | 22:0 |
|-----------------|------|------|-------|------|--------------|---------------|-----------------|------------------|------|------|------|
| | | | | | $\Delta 6,9$ | $\Delta 9,12$ | $\Delta 6,9,12$ | $\Delta 9,12,15$ | | | |
| % | % | % | % | % | % | % | % | % | % | % | % |
| 3.89 | 0.25 | 1.19 | 67.73 | 0 | 22.46 | 0.1 | 1.97 | 0 | 0.54 | 1.32 | 0.44 |
| LP30108 control | | | | | | | | | | | |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1333 | 64 | 5544-LP30108-20 | 6.53 | 0.15 | 0.98 | 23.33 | 0.01 | 21.1 | 43.3 | 1.34 | 0.84 | 0.52 | 0.97 |
| 97XX1333 | 65 | 5544-LP30108-20 | 6.9 | 0.29 | 1.17 | 8.89 | 0.03 | 15.07 | 60.5 | 1.12 | 2.23 | 0.98 | 0.86 |
| 97XX1333 | 66 | 5544-LP30108-20 | 8.15 | 0.2 | 3.6 | 16.87 | 0.11 | 16.05 | 48.23 | 1.1 | 1.18 | 1.71 | 0.66 |
| 97XX1333 | 67 | 5544-LP30108-20 | 8.85 | 0.35 | 1.2 | 14.49 | 0.01 | 25.66 | 43.98 | 1.8 | 1.03 | 0.65 | 0.76 |
| 97XX1333 | 68 | 5544-LP30108-20 | 6.05 | 0.16 | 1.27 | 17.85 | 0.16 | 16.13 | 53.16 | 1.14 | 1.25 | 0.71 | 0.85 |
| 97XX1333 | 69 | 5544-LP30108-20 | 7.16 | 0.21 | 1.33 | 11.51 | 0.09 | 17.42 | 56.13 | 1.41 | 1.58 | 0.93 | 0.68 |
| 97XX1333 | 70 | 5544-LP30108-20 | 3.46 | 0.04 | 1.76 | 18.38 | 0.03 | 22.55 | 48.55 | 1.22 | 1.04 | 0.83 | 0.95 |
| 97XX1333 | 71 | 5544-LP30108-20 | 3.71 | 0.05 | 1.74 | 16.11 | 0.01 | 26.93 | 45.79 | 1.47 | 1.02 | 0.89 | 1 |
| 97XX1333 | 72 | 5544-LP30108-20 | 3.5 | 0.04 | 1.76 | 23.74 | 0.02 | 35.38 | 30.82 | 1.87 | 0.58 | 0.65 | 0.89 |
| 97XX1333 | 73 | 5544-LP30108-20 | 4.67 | 0.11 | 1.87 | 17.98 | 0.04 | 22.47 | 47.89 | 1.17 | 0.89 | 0.93 | 0.88 |
| 97XX1333 | 74 | 5544-LP30108-20 | 4.52 | 0.09 | 1.86 | 13.77 | 0.03 | 20.9 | 52.96 | 1.31 | 1.19 | 1.03 | 0.88 |
| 97XX1333 | 75 | 5544-LP30108-20 | 5.26 | 0.13 | 1.64 | 16.46 | 0.05 | 21.75 | 49.42 | 1.25 | 1.08 | 0.83 | 0.86 |
| 97XX1333 | 76 | 5544-LP30108-20 | 7.61 | 0.21 | 1.44 | 12.49 | 0.33 | 17 | 55.31 | 1.18 | 1.59 | 0.88 | 0.74 |
| 97XX1333 | 77 | 5544-LP30108-20 | 6.42 | 0.15 | 1.51 | 10.79 | 0.09 | 15.96 | 58.77 | 1.12 | 1.53 | 0.98 | 0.85 |
| 97XX1333 | 78 | 5544-LP30108-20 | 4.59 | 0.16 | 0.93 | 12.1 | 0.08 | 15.94 | 60.15 | 1.12 | 1.69 | 0.74 | 0.88 |
| 97XX1333 | 79 | 5544-LP30108-20 | 5.24 | 0.09 | 1.94 | 14.08 | 0.21 | 19.79 | 53.58 | 1.05 | 1.03 | 0.96 | 0.84 |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1333 | 80 | 5544-LP30108-20 | 4.38 | 0.08 | 1.66 | 22.25 | 0 | 30.79 | 35.49 | 2.16 | 0.72 | 0.66 | 0.84 |
| 97XX1333 | 81 | 5544-LP30108-20 | 4.05 | 0.05 | 1.44 | 24.16 | 0.04 | 24.86 | 40.89 | 1.42 | 0.79 | 0.63 | 0.84 |
| 97XX1333 | 82 | 5544-LP30108-20 | 3.29 | 0.05 | 1.9 | 19.66 | 0 | 23.83 | 46.48 | 1.27 | 0.87 | 0.78 | 0.81 |
| 97XX1333 | 83 | 5544-LP30108-20 | 4.82 | 0.08 | 1.99 | 17.27 | 0.1 | 20.69 | 49.73 | 1.22 | 1.06 | 0.98 | 0.82 |
| 97XX1333 | 84 | 5544-LP30108-20 | 5.33 | 0.1 | 1.77 | 13.6 | 0.03 | 21.44 | 51.74 | 1.52 | 1.21 | 0.98 | 0.93 |
| 97XX1333 | 85 | 5544-LP30108-20 | 3.3 | 0.05 | 1.2 | 68.23 | 0 | 22.09 | 0.01 | 2.27 | 0 | 0.57 | 1.57 |
| 97XX1333 | 86 | 5544-LP30108-20 | 3.23 | 0.05 | 1.54 | 28.15 | 0.01 | 36.4 | 25.91 | 1.99 | 0.43 | 0.59 | 0.97 |
| 97XX1333 | 87 | 5544-LP30108-20 | 4.38 | 0.1 | 1.16 | 60.94 | 2.85 | 8.35 | 17.61 | 1.26 | 0.69 | 0.54 | 1.39 |
| 97XX1333 | 88 | 5544-LP30108-20 | 4.4 | 0.09 | 1.34 | 38.42 | 0.02 | 34.74 | 16.61 | 2.12 | 0.32 | 0.53 | 0.82 |
| 97XX1278 | 16 | 5544-LP30108-15 | 3.62 | 0.11 | 1.22 | 27.23 | 0 | 30.9 | 32.87 | 1.41 | 0.48 | 0.46 | 0.97 |
| 97XX1278 | 17 | 5544-LP30108-15 | 3.68 | 0.13 | 1.26 | 45.29 | 0 | 44.79 | 0.72 | 1.77 | 0.01 | 0.43 | 1.24 |
| 97XX1278 | 18 | 5544-LP30108-15 | 4.08 | 0.15 | 1.49 | 22.34 | 0 | 28.37 | 39.37 | 1.22 | 0.64 | 0.55 | 0.88 |
| 97XX1278 | 19 | 5544-LP30108-15 | 3.51 | 0.1 | 1.01 | 35.44 | 0 | 44.12 | 11.7 | 1.72 | 0.15 | 0.36 | 1.14 |
| 97XX1278 | 20 | 5544-LP30108-15 | 3.66 | 0.12 | 1.21 | 27.44 | 0 | 30.2 | 32.37 | 1.49 | 0.53 | 0.49 | 1.15 |
| 97XX1278 | 21 | 5544-LP30108-15 | 3.58 | 0.11 | 1.51 | 29.81 | 0 | 30.72 | 30.65 | 1.16 | 0.4 | 0.5 | 0.96 |
| 97XX1278 | 23 | 5544-LP30108-15 | 3.69 | 0.11 | 1.42 | 30.05 | 0 | 32.28 | 27.41 | 1.65 | 0.38 | 0.54 | 1.19 |
| 97XX1278 | 24 | 5544-LP30108-15 | 3.56 | 0.11 | 1.31 | 30.25 | 0 | 28.64 | 31.46 | 1.43 | 0.48 | 0.48 | 1.11 |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1278 | 25 | 5544-LP30108-15 | 4.41 | 0.22 | 2.08 | 15.05 | 0 | 23.77 | 49.51 | 1.18 | 0.96 | 0.87 | 0.85 |
| 97XX1278 | 26 | 5544-LP30108-15 | 3.75 | 0.14 | 1.59 | 23.55 | 0 | 27.91 | 38.8 | 1.39 | 0.61 | 0.59 | 0.97 |
| 97XX1278 | 27 | 5544-LP30108-15 | 3.67 | 0.11 | 1.9 | 26.07 | 0 | 31.1 | 33.16 | 1.08 | 0.49 | 0.65 | 0.97 |
| 97XX1278 | 28 | 5544-LP30108-15 | 3.82 | 0.11 | 1.54 | 21.27 | 0 | 29.07 | 39.69 | 1.47 | 0.7 | 0.58 | 0.86 |
| 97XX1278 | 29 | 5544-LP30108-15 | 3.65 | 0.14 | 1.27 | 45.84 | 0 | 43.38 | 1 | 2.33 | 0.02 | 0.42 | 1.27 |
| 97XX1278 | 30 | 5544-LP30108-15 | 3.59 | 0.12 | 1.19 | 30.41 | 0 | 30.68 | 30.37 | 1.24 | 0.4 | 0.37 | 0.99 |
| 97XX1278 | 31 | 5544-LP30108-15 | 3.74 | 0.12 | 1.26 | 38.98 | 0 | 50.53 | 0.98 | 2.12 | 0.02 | 0.39 | 1.14 |
| 97XX1278 | 32 | 5544-LP30108-15 | 3.86 | 0.11 | 1.46 | 26.38 | 0 | 28.9 | 35.41 | 1.01 | 0.5 | 0.54 | 0.97 |

Example 11

Simultaneous expression of *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases in *Brassica napus*

5 In order to produce arachadonic acid (ARA) in transgenic canola oil both $\Delta 5$ and $\Delta 6$ desaturase activities need to be introduced. In order to facilitate downstream characterization and breeding, it may be advantageous to have both activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of $\Delta 5$ and $\Delta 6$ desaturases.

10 The Asp718 fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5545. The NotI fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5545
15 to create pCGN5546. The expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma29 and the nptII marker is the same.

 PCGN5546 was introduced into *Brassica napus* cv.LP30108 via
20 *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 12. All the lines show expression of both desaturases as evidenced by the presence of $\Delta^{5,9}$ 18:2 (as seen in pCGN5531 plants) and $\Delta^{6,9}$ 18:2 and GLA (as seen in pCGN5538 plants)

25 .

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5,9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|-----------------|------|------|------|-------|-----------|-----------|------------|------------------|-------------------|------|------|------|
| 5546-LP30108-1 | 4.88 | 0.33 | 2.28 | 57.2 | 4.68 | 6.08 | 7.36 | 12.29 | 1.38 | 0.85 | 0.84 | 1.22 |
| 5546-LP30108-2 | 4.01 | 0.14 | 2.22 | 66.04 | 2.73 | 1.33 | 12.6 | 6.45 | 1.41 | 0.32 | 0.75 | 1.2 |
| 5546-LP30108-3 | 4.29 | 0.15 | 2.55 | 68.89 | 0.44 | 0.58 | 16.97 | 1.66 | 1.6 | 0.11 | 0.88 | 1.22 |
| 5546-LP30108-4 | 4.24 | 0.14 | 2.6 | 70.48 | 0.73 | 0.52 | 14.28 | 2.61 | 1.42 | 0.14 | 0.96 | 1.26 |
| 5546-LP30108-5 | 3.52 | 0.15 | 2.01 | 60.3 | 1.72 | 0.95 | 16.92 | 9.88 | 1.66 | 0.39 | 0.68 | 1.26 |
| 5546-LP30108-6 | 4.05 | 0.17 | 2.24 | 61.29 | 1.98 | 0.4 | 18.87 | 6.28 | 2 | 0.34 | 0.7 | 1.24 |
| 5546-LP30108-7 | 4.74 | 0.21 | 2.49 | 64.5 | 2.25 | 1.18 | 10.03 | 9.73 | 1.35 | 0.52 | 0.97 | 1.28 |
| 5546-LP30108-8 | 4.24 | 0.14 | 2.82 | 63.92 | 1.9 | 1.5 | 11.67 | 9.29 | 1.44 | 0.43 | 0.89 | 1.19 |
| 5546-LP30108-9 | 3.8 | 0.13 | 2.15 | 65.75 | 2.3 | 0.16 | 14.92 | 6.32 | 1.57 | 0.24 | 0.75 | 1.35 |
| 5546-LP30108-10 | 4.28 | 0.17 | 1.55 | 58.8 | 1.1 | 0.12 | 22.95 | 5.97 | 2.24 | 0.22 | 0.6 | 1.35 |
| 5546-LP30108-11 | 4.25 | 0.15 | 1.82 | 63.68 | 1.01 | 0.22 | 19.42 | 4.96 | 1.81 | 0.2 | 0.67 | 1.23 |
| 5546-LP30108-12 | 3.95 | 0.14 | 2.36 | 66.9 | 1.12 | 0.01 | 19.42 | 1.59 | 1.77 | 0.04 | 0.8 | 1.21 |
| 5546-LP30108-13 | 4.18 | 0.16 | 2.17 | 66.91 | 1.36 | 0.02 | 18.84 | 1.99 | 1.74 | 0.05 | 0.77 | 1.15 |
| 5546-LP30108-14 | 4.74 | 0.26 | 1.82 | 65.29 | 1.25 | 0.27 | 16.77 | 5.3 | 1.59 | 0.25 | 0.71 | 1.32 |
| 5546-LP30108-15 | 4.3 | 0.23 | 2.54 | 65.65 | 1.67 | 0.59 | 13.15 | 7.22 | 1.54 | 0.36 | 0.88 | 1.3 |
| 5546-LP30108-16 | 4.05 | 0.17 | 2.75 | 64.13 | 2.56 | 2.8 | 9.56 | 9.31 | 1.34 | 0.53 | 0.92 | 1.28 |

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5,9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|-----------------|------|------|------|-------|-----------|-----------|------------|------------------|-------------------|------|------|------|
| 5546-LP30108-17 | 4.06 | 0.13 | 2.85 | 65.76 | 2.09 | 1.92 | 9.65 | 9.1 | 1.23 | 0.45 | 0.92 | 1.22 |
| 5546-LP30108-18 | 4.16 | 0.25 | 2.14 | 60.68 | 1.43 | 0.02 | 24.02 | 2.62 | 2.11 | 0.09 | 0.69 | 1.26 |
| 5546-LP30108-19 | 5.77 | 0.37 | 2.15 | 56.11 | 1.6 | 0.33 | 19.34 | 9.16 | 2.37 | 0.46 | 0.73 | 1.05 |
| 5546-LP30108-20 | 5.03 | 0.36 | 2.34 | 61.05 | 1.55 | 0.35 | 17.21 | 6.96 | 2.24 | 0.39 | 0.77 | 1.22 |
| 5546-LP30108-21 | 4.52 | 0.3 | 2.71 | 62.14 | 1.33 | 0.23 | 17.62 | 6.44 | 1.88 | 0.28 | 0.88 | 1.15 |
| 5546-LP30108-22 | 5.91 | 0.44 | 2.15 | 60.12 | 1.41 | 0.36 | 17.04 | 7.75 | 1.97 | 0.36 | 0.78 | 1.07 |
| 5546-LP30108-23 | 4.28 | 0.22 | 2.44 | 66.19 | 0.93 | 0.11 | 17.03 | 4.37 | 1.67 | 0.17 | 0.82 | 1.25 |
| 5546-LP30108-24 | 4.92 | 0.33 | 2.68 | 62.6 | 1.32 | 0.36 | 16.89 | 5.82 | 2.05 | 0.3 | 0.95 | 1.19 |
| 5546-LP30108-25 | 5.42 | 0.72 | 3.15 | 47.47 | 2.66 | 4.21 | 13.51 | 16.31 | 2.14 | 0.99 | 1.18 | 1.37 |
| 5546-LP30108-26 | 3.85 | 0.22 | 2.78 | 65.02 | 1.05 | 0.05 | 18.35 | 4.36 | 1.67 | 0.12 | 0.82 | 1.18 |
| 5546-LP30108-27 | 3.86 | 0.15 | 2.76 | 65.17 | 1.11 | 0.78 | 16.24 | 5.21 | 1.53 | 0.25 | 0.93 | 1.3 |
| 5546-LP30108-28 | 5.29 | 0.42 | 1.81 | 49.12 | 1.07 | 0.09 | 30.52 | 5.21 | 3.57 | 0.44 | 0.67 | 1.23 |
| 5546-LP30108-29 | 4.4 | 0.2 | 2.38 | 65.95 | 1.05 | 0.28 | 16.31 | 4.85 | 1.64 | 0.19 | 0.85 | 1.26 |
| 5546-LP30108-30 | 3.99 | 0.19 | 2.55 | 67.47 | 0.83 | 0.11 | 17.02 | 3.18 | 1.68 | 0.13 | 0.83 | 1.23 |

Example 12

Simultaneous expression of *M. alpina* $\Delta 5$, $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5 In order to achieve optimal production of ARA in transgenic canola oil both the $\Delta 6$ and $\Delta 12$ desaturase activities may need to be present in addition to the $\Delta 5$ activity. In order to facilitate downstream characterization and breeding, it may be advantageous to have all of these activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of $\Delta 5$, $\Delta 6$ and $\Delta 12$ desaturases.

10 The HindIII fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the HindIII site of pCGN5544 to create pCGN5547. The expression modules were oriented in such a way that the direction of transcription from Ma29, Ma524, Ma648 and the nptII marker is the same.

15 PCGN5547 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC.

20 The results are shown in Table 13. Twenty-seven of the lines show significant accumulation of GLA and in general the levels of GLA observed are higher than those seen in the 5546 plants that did not contain the $\Delta 12$ desaturase. The $\Delta 12$ desaturase appears to be active in most lines as evidenced by the lack of detectable $\Delta 6,9$ 18:2 and elevated 18:2 levels in most plants. Small amounts of

25 $\Delta 5,9$ 18:2 are seen in the 5547 plants, although the levels are generally less than those observed in the 5546 plants. This may be due to the presence of the $\Delta 12$ desaturase which efficiently converts the 18:1 to 18:2 before it can be desaturated at the $\Delta 5$ position.

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

| STRAIN ID | 12:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5, 9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 | 22:1 | 22:2 |
|-----------------|------|------|------|------|-------|---------------|-----------|------------|------------------|-------------------|------|------|------|------|------|
| 5547-LP30108-1 | 0.0 | 5.38 | 0.3 | 2.23 | 64.12 | 0.01 | 0 | 22.67 | 0.44 | 2.17 | 0.07 | 0.82 | 1.11 | 0.03 | 0 |
| 5547-LP30108-2 | 0.1 | 4.45 | 0.13 | 2.29 | 51.57 | 0.16 | 0 | 33.85 | 3.18 | 1.74 | 0.03 | 0.78 | 1.02 | 0.03 | 0.02 |
| 5547-LP30108-3 | 0.0 | 4.18 | 0.12 | 2.03 | 59.61 | 0.03 | 0 | 29.44 | 0.44 | 1.64 | 0 | 0.75 | 1.15 | 0.03 | 0.01 |
| 5547-LP30108-4 | 0.0 | 4.35 | 0.15 | 2.29 | 50.59 | 0.12 | 0.01 | 37.31 | 0.85 | 1.86 | 0.02 | 0.78 | 1.02 | 0.02 | 0.01 |
| 5547-LP30108-5 | 0.0 | 4.59 | 0.14 | 1.83 | 49 | 0.25 | 0.01 | 31.65 | 8.16 | 1.86 | 0.13 | 0.68 | 1.04 | 0.02 | 0 |
| 5547-LP30108-6 | 0.0 | 4.11 | 0.15 | 2.53 | 44.3 | 0.13 | 0 | 28.12 | 15.89 | 1.94 | 0.28 | 0.82 | 1.13 | 0 | 0 |
| 5547-LP30108-7 | 0.0 | 4.27 | 0.15 | 2.55 | 39.18 | 0.12 | 0.02 | 27 | 21.72 | 1.87 | 0.45 | 0.89 | 1.08 | 0 | 0 |
| 5547-LP30108-8 | 0.0 | 4.3 | 0.14 | 2.92 | 42.83 | 0.26 | 0 | 30.81 | 14.51 | 1.49 | 0.22 | 0.89 | 1.06 | 0 | 0 |
| 5547-LP30108-9 | 0.0 | 4.46 | 0.17 | 3.13 | 44.51 | 0 | 0 | 30.12 | 12.87 | 1.76 | 0.22 | 0.98 | 1.12 | 0.01 | 0 |
| 5547-LP30108-10 | 0.0 | 4.28 | 0.11 | 2.62 | 41.44 | 0.28 | 0 | 30.89 | 16.28 | 1.45 | 0.21 | 0.82 | 1.06 | 0 | 0 |
| 5547-LP30108-11 | 0.0 | 4.47 | 0.17 | 2.43 | 26.96 | 0.48 | 0 | 34.44 | 25.01 | 2.14 | 0.63 | 0.84 | 0.99 | 0 | 0 |
| 5547-LP30108-12 | 0.0 | 4.36 | 0.16 | 2.68 | 42.2 | 0.17 | 0 | 29.78 | 15.93 | 1.83 | 0.27 | 0.88 | 1.06 | 0 | 0 |
| 5547-LP30108-13 | 0.0 | 4.87 | 0.19 | 2.81 | 21.7 | 0.53 | 0 | 32.83 | 30.54 | 2.04 | 0.8 | 1 | 0.89 | 0.02 | 0.01 |
| 5547-LP30108-14 | 0.0 | 4.61 | 0.25 | 2.6 | 54 | 0 | 0 | 32.98 | 0.5 | 2.46 | 0.03 | 0.86 | 1.14 | 0 | 0 |
| 5547-LP30108-15 | 0.0 | 4.07 | 0.14 | 2.98 | 37.09 | 0.14 | 0.01 | 29.01 | 21.55 | 1.66 | 0.38 | 1.06 | 1.11 | 0 | 0 |

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

| STRAIN ID | 12:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5, 9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 | 22:1 | 22:2 |
|-----------------|------|------|------|------|-------|---------------|-----------|------------|------------------|-------------------|------|------|------|------|------|
| 5547-LP30108-16 | 0.0 | 3.63 | 0.13 | 2.12 | 64.69 | 0 | 0 | 24.21 | 0.15 | 2.04 | 0 | 0.82 | 1.56 | 0.02 | 0 |
| 5547-LP30108-17 | 0.0 | 3.85 | 0.18 | 2.22 | 67.22 | 0.01 | 0 | 21.25 | 0 | 2.27 | 0 | 0.83 | 1.53 | 0 | 0 |
| 5547-LP30108-18 | 0.0 | 5.46 | 0.19 | 2.87 | 41.83 | 0.1 | 0.04 | 22.76 | 21.45 | 1.72 | 0.48 | 1.06 | 1.23 | 0 | 0 |
| 5547-LP30108-19 | 0.0 | 4.33 | 0.12 | 2.73 | 50.31 | 0.07 | 0 | 24.77 | 12.72 | 1.62 | 0.21 | 1.04 | 1.29 | 0 | 0.01 |
| 5547-LP30108-20 | 0.0 | 4.22 | 0.12 | 2.91 | 46.33 | 0.25 | 0 | 26.87 | 14.65 | 1.61 | 0.22 | 0.98 | 1.18 | 0 | 0 |
| 5547-LP30108-21 | 0.0 | 4.38 | 0.17 | 2.37 | 55.37 | 0 | 0 | 32.59 | 0.53 | 1.85 | 0.03 | 0.83 | 1.23 | 0 | 0 |
| 5547-LP30108-22 | 0.0 | 5.5 | 0.18 | 2.71 | 41.93 | 0.1 | 0.19 | 24.19 | 20.14 | 1.76 | 0.45 | 0.94 | 1.21 | 0 | 0 |
| 5547-LP30108-23 | 0.0 | 4.03 | 0.16 | 2.17 | 68.44 | 0 | 0 | 20.09 | 0 | 2.19 | 0.02 | 0.83 | 1.46 | 0 | 0 |
| 5547-LP30108-24 | 0.0 | 4.19 | 0.17 | 2.72 | 49.31 | 0 | 0 | 30.38 | 8.64 | 1.85 | 0.13 | 0.86 | 1.16 | 0 | 0 |
| 5547-LP30108-25 | 0.0 | 4.04 | 0.17 | 2.1 | 70.48 | 0 | 0 | 18.04 | 0.05 | 2.09 | 0 | 0.86 | 1.54 | 0 | 0 |
| 5547-LP30108-26 | 0.0 | 4.74 | 0.22 | 3.2 | 26.74 | 0.33 | 0 | 30.05 | 28.95 | 2.02 | 0.78 | 1.08 | 0.99 | 0 | 0 |
| 5547-LP30108-27 | 0.0 | 4.29 | 0.18 | 2.23 | 52.49 | 0 | 0 | 28.48 | 7.36 | 1.91 | 0.13 | 0.87 | 1.37 | 0 | 0 |
| 5547-LP30108-28 | 0.0 | 4.36 | 0.17 | 3 | 44.35 | 0.2 | 0 | 29.59 | 13.39 | 1.91 | 0.23 | 0.96 | 1.17 | 0 | 0 |
| 5547-LP30108-29 | 0.0 | 4.32 | 0.17 | 2.94 | 52.53 | 0.05 | 0 | 33.88 | 0.91 | 2.34 | 0.01 | 0.97 | 1.23 | 0 | 0 |
| 5547-LP30108-30 | 0.0 | 4.07 | 0.14 | 2.89 | 45.13 | 0.01 | 0 | 29.06 | 13.96 | 1.71 | 0.2 | 0.94 | 1.2 | 0.01 | 0 |

Example 13

Stereospecific Distribution of $\Delta 6$ -Desaturated Oils

This experiment was designed to investigate the stereospecific distribution of the $\Delta 6$ -desaturated oils in seeds expressing pCGN5538 (Ma 524 cDNA). Three seed samples were used:

- 1) Non-transformed *B. napus* cv. LP004 seeds (control)
- 2) Segregating T2 seeds of pCGN5538-LP004-19
- 3) Segregating T2 seeds of pCGN5538-LP004-29

The following protocol was used for the analysis:

1. Seed Oil Extraction

Fifty seeds were placed in a 12 x 32 mm vial and crushed with a glass rod. 1.25 mL hexane was added and the mixture was vortexed. The seeds were extracted overnight on a shaker. The extract was then filtered through a 0.2 micron filter attached to a 1cc syringe. The extract was then dried down under nitrogen. The resulting oil was used for digestion and derivatization of the whole oil sample.

2. Digestion

A. Liquid Oil Digestion

The stock lipase (from *Rhizopus arrhizus*, Sigma, L4384) was diluted to approximately 600,000 units/mL with a goal of obtaining 50% digestion of the TAG. The stock lipase is maintained at 4 degrees C and placed on ice. The amount of reagents may be adjusted according to the amount of oil to be digested.

The following amounts are based on a 2.0 mg extracted oil sample. In a 12 x 32 mm screw cap vial the following were added: 2.0 mg oil, 200 μ L 0.1 M tris HCl pH 7, 40 μ L 2.2 w/v% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 100 μ L 0.05 w/v % bile salts. The material was vortexed and sonicated to disperse the oil. Twenty μ L of diluted lipase was added and the mixture was vortexed continuously for 1.0

minute at room temperature. A white precipitate formed. The reaction was stopped with 100 μ L 6M HCl and vortexing. Five hundred μ L CHCl_3 : CH_3OH (2:1) was added and the mixture was vortexed and held on ice while reaining digestions were carried out. Samples were vortexed again and centrifuged
5 briefly to sharpen layers. The lower layer containing digest products was removed with a pasteur pipette and placed in a 12 x 32 mm crimp cap vial. The material was then re-extracted with 300 μ L CHCl_3 , vortexed, centrifuged, and combined with the lower layers. The digest products were kept on ice as much as possible. HPLC separation is performed as soon as possible after digestion to
10 minimize acyl migration.

B. Solid Fat Digestion

The procedure for liquid oil digestion described above was followed except that 20 μ L 11:0 methyl ester is added to 2.0 mg solid fat.

3. HPLC Separation

15 The digestion products were dried down in chloroform to approximately 200 μ L. Each sample was then transferred into an insert in an 8 x 40 mm shell vial and 30 μ L was injected for HPLC analysis.

20 The high performance liquid chromatographic system was equipped with a Varex ELSD IIA evaporative light scattering detector with tube temperature at 105°C and nitrogen gas flow at 40 mL/min; a Waters 712 Wisp autosampler, three Beckman 114M Solvent Delivery Modules; a Beckman 421A controller, a Rheodyne pneumatically actuated stream splitter; and a Gilson micro fractionator. The chromatography column is a 220 x 4.6 mm, 5 micron normal phase silica cartridge by Brownlee.

25 The three solvents used were:

A= hexane:toluene 1:1

B= toluene: ethyl acetate 3:1

C= 5% formic acid in ethyl acetate

The gradient profile was as follows:

| Time (min) | Function | Value | Duration |
|------------|-------------|-------|----------|
| 0 flow | 2.0 mL/min | | |
| 0 % B | 10 | | |
| 0 % C | 2 | | |
| 2 % C | 25 | | 6 min |
| 14.0 % C | 2 | | 1 min |
| 15.0 | End program | | |

A chromatographic standard mixture is prepared in hexane:toluene 1:1 containing the following:

- 0.2 mg/mL triglyceride 16:0
- 5 2.0 mg/mL 16:0 Free Fatty Acid
- 0.2 mg/mL di16:0 mixed isomers (1,2-diacylglycerol and 1,3-diacylglycerol)
- 0.2 mg/mL 3-mono acylglycerol 16:0
- 0.2 mg/mL 2-mono acylglycerol 16:0

10 For each sample, the fraction containing the 2-mag peak is collected automatically by method controlled timed events relays. A time delay is used to synchronize the detector with the collector's emitter. The 2-mag peaks are collected and the fractions are evaporated at room temperature overnight.

15 The *sn*-2 composition results rely on minimization of acyl migration. Appearance of 1-monoacylglycerol and/or 3-monoacylglycerol peaks in the chromatograph means that acyl migration has occurred.

4. Derivatization

20 To derivatize the whole oil, 1.0 mg of the extracted whole oil was weighed into a 12 x 32 mm crimp cap vial. One mL toluene was then added. The sample is then vortexed and a 50 μ L aliquot was removed for derivatization. To the dried down 2-mag samples, 50 μ L toluene was added. To both the whole oil and 2-mag fractions 105 μ L H₂SO₄/CH₃OH @ 8.76 wt% is added. The cap was tightly capped and the sample is refluxed for 1 hour at 95 degrees C. The sample was allowed to cool and 500 μ L 10 w/v % NaCl in

water and 60 uL heptane was added. The organic layer was removed and inserted in a 12 x 32 mm crimp cap vial.

5. GLC Analysis

5 A Hewlett Packard model 6890 GC equipped with a split/splitless capillary inlet, FID detector, 6890 series autosampler and 3392A Alpha Omega integrator is set up for the capillary column as follows:

A. Supelco Omegawax 250, 30 m length, 0.25 mm id, 0.25 um film thickness

| | | |
|----|-----------------|----------------|
| 10 | injection port: | 260 C |
| | detector: | 270 C |
| | initial temp: | 170 C |
| | initial time: | 1.5 min |
| | rate: | 30 deg/min |
| 15 | final temp: | 245 C |
| | final time: | 6.5 min |
| | injection vol: | 1.5 uL |
| | head pressure: | 25 psi |
| | split ratio: | 30 |
| 20 | carrier gas: | He |
| | make-up gas: | N ₂ |
| | FID gas: | H + air |

Percent compositions of fatty acid methyl esters are calculated as mole percents. For carbon chain lengths less than 12, the use of theoretical or
25 empirical response factors in the area percent calculation is desirable.

6. Calculations

The mean distribution of each acyl group at each *sn*-1 and *sn*-3 position was calculated.

mean *sn*-1 and *sn*-3 composition = (3 WO comp - MAG comp) / 2

5 WO = whole oil

MAG= monoacylglycerol

The results of this analysis are presented in Table 14. The GLA and $\Delta^{6,9}$ 18:2 are evenly distributed between the *sn*-2 and *sn*-1, 3 positions. This analysis can not discriminate between fatty acids in the *sn*-1 vs. *sn*-3 positions.

Table 14

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2 | 18:3_Δ6,9,12 | 8:3 | 18:4 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|-------|--------------|------|------|------|------|
| Control | | | | | | | | | | | |
| sn2 composition | 1.23 | 0.15 | 0.37 | 64.77 | 0.00 | 29.45 | 0.06 | 2.01 | 0.00 | 0.21 | 0.57 |
| whole oil composition | 4.33 | 0.20 | 3.32 | 69.29 | 0.18 | 18.51 | 0.00 | 1.35 | 0.06 | 0.91 | 1.17 |
| mean sn1, sn3 composition* | 5.88 | 0.23 | 4.80 | 71.55 | 0.27 | 13.04 | -0.03 | 1.02 | 0.09 | 1.26 | 1.47 |
| | | | | | | | | | | | |
| 5538-19 | | | | | | | | | | | |
| sn2 composition | 1.65 | 0.27 | 4.12 | 57.21 | 5.61 | 14.55 | 12.45 | 1.38 | 0.32 | 0.43 | 1.00 |
| whole oil composition | 5.44 | 0.33 | 4.09 | 57.51 | 4.53 | 10.57 | 13.16 | 1.03 | 0.50 | 1.07 | 1.07 |
| mean sn1, sn3 composition* | 7.34 | 0.36 | 4.08 | 57.66 | 3.99 | 8.58 | 13.52 | 0.86 | 0.59 | 1.39 | 1.11 |
| | | | | | | | | | | | |
| 5538-29 | | | | | | | | | | | |
| sn2 composition | 1.24 | 0.27 | 1.56 | 56.35 | 6.35 | 17.85 | 12.99 | 1.60 | 0.38 | 0.14 | 0.40 |
| whole oil composition | 4.96 | 0.32 | 3.73 | 54.92 | 4.99 | 12.11 | 13.66 | 1.10 | 0.50 | 0.99 | 1.11 |
| mean sn1, sn3 composition* | 6.82 | 0.35 | 4.82 | 54.21 | 4.31 | 9.24 | 14.00 | 0.85 | 0.56 | 1.42 | 1.47 |
| | | | | | | | | | | | |
| *calculated from the mag and whole oil composition for each analyte | | | | | | | | | | | |

Example 14

Fatty Acid Compositions of Transgenic Plants

$\Delta 5$ and $\Delta 6$ transgenic plants were analyzed for their fatty acid content.

The following protocol was used for oil extraction:

- 5 1. About 400 mg of seed were weighed out in duplicate for each sample.
2. The seeds were crushed in a mortar and pestle. The mortar and pestle was rinsed twice with 3ml (2:1) (v:v) $\text{CHCl}_3:\text{CH}_3\text{OH}/\text{MeOH}$. An additional 6 ml (2:1) was added to
10 the 20ml glass vial (oil extracted in 12ml total 2:1).
3. Samples were vortexed and placed on an orbital shaker for 2 hours with occasional vortexing.
4. 5ml of 1M NaCl was added to each sample. Sample was vortexed then spun in centrifuge at 2000rpm for 5 minutes.
15 Lower phase was drawn off using a pasteur pipette.
5. Upper phase was re-extracted with an additional 5ml. Sample was vortexed then spun in centrifuge at 2000 rpm for 5 minutes. The lower phase was drawn off using a pasteur pipette and added to previous lower phase.
- 20 6. $\text{CHCl}_3:\text{CH}_3\text{OH}/\text{MeOH}$ was evaporated under nitrogen using evaporative cooling. Vial containing extracted oil was sealed under nitrogen. Between 120mg- 160mg oil was extracted for each sample.

25 For GC-MS analysis, fatty acid methyl esters were dissolved in an appropriate volume of hexane and analyzed using a Hewlett-Packard 5890 Series II Plus gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a 30 m x 0.32 mm i.d. Omegawax 320 fused silica capillary column (Supelco, Bellefonte, PA) and a Hewlett-Packard 5972 Series mass selective detector. Mass spectra were interpreted by comparison to the mass spectra in

NIST/EPA/NIH Chemical Structure Database using a MS Chem Station
(#G1036A) (Hewlett Packard).

Transgenic line 5531-6 was analyzed in duplicate (A, B) and compared
to control line LP004-6. The fatty acid profile results are shown in Table 15.

- 5 Transgenic line 5538-19 was analyzed in duplicate (A, B) and compared
to control line LP004-6. The fatty acid profile results are shown in Table 16.

Table 15
Fatty Acid Profile

| | CONTROL | CONTROL | TRANSGENIC | TRANSGENIC |
|--------------|------------|------------|------------|------------|
| | | | | |
| | LP004-6A | LP004-6B | 5531-6A | 5531-6B |
| | | | | |
| | LRL-2043 | LRL-2044 | LRL-2042 | LRL-2045 |
| | 001f0102.d | 001f0103.d | 001f0101.d | 001f0104.d |
| C12:0 | | | | |
| C13:0 | | | | |
| C14:0 | | 0.053 | | 0.061 |
| C14:1 | | | | |
| C15:0 isomer | | | | |
| C15:0 | | | | |
| C16:0 | 4.107 | 4.034 | 4.257 | 4.224 |
| C16:1 | 0.181 | 0.173 | 0.200 | 0.199 |
| C16:2 | 0.061 | 0.065 | 0.081 | 0.060 |
| C17:0 | | | | |
| C16:3 | 0.244 | 0.246 | 0.155 | 0.151 |
| C16:4 | | | | |
| C18:0 | 2.608 | 2.714 | 3.368 | 3.417 |
| C18:1w9 | 65.489 | 66.454 | 59.529 | 59.073 |
| C18:1w7 | 2.297 | 2.185 | 2.388 | 2.393 |
| C18:2 5,9 | | | 6.144 | 6.269 |
| C18:2w6 | 19.828 | 18.667 | 18.872 | 19.059 |
| C18:3 5,9,12 | | | 0.469 | 0.496 |
| C18:3w6 | | 0.060 | | |
| C18:3w3 | 1.587 | 1.578 | 1.428 | 1.418 |
| C18:4w6 | | | | |
| C18:4w3 | | | | |
| C20:0 | 0.962 | 0.998 | 1.009 | 1.022 |
| C20:1w11 | 1.336 | 1.335 | 1.058 | 1.065 |
| C20:1w9 | | | | |
| C20:1w7 | | | 0.076 | 0.080 |
| C20:2w6 | 0.073 | 0.073 | | 0.052 |
| C20:3w6 | | | | |

Table 15
Fatty Acid Profile

| | CONTROL | CONTROL | TRANSGENIC | TRANSGENIC |
|---------------------|-------------------|-------------------|-------------------|-------------------|
| | | | | |
| | LP004-6A | LP004-6B | 5531-6A | 5531-6B |
| | | | | |
| | LRL-2043 | LRL-2044 | LRL-2042 | LRL-2045 |
| | 001f0102.d | 001f0103.d | 001f0101.d | 001f0104.d |
| C20:4w6 | | | | |
| C20:3w3 | | | | |
| C20:4w3 | | | | |
| C20:5w3 | | | | |
| C22:0(1.000) | 0.542 | 0.558 | 0.463 | 0.467 |
| C22:1w11 | | 0.038 | | |
| C22:1w9 | | | | |
| C22:1w7 | | 0.034 | | |
| C21:5 | | | | |
| C23:0 | | 0.029 | | |
| C22:4w6 | | | | |
| C22:5w6 | | | | |
| C22:5w3 | | | | |
| C24:0 | 0.373 | 0.391 | 0.280 | 0.283 |
| C22:6w3 | 0.314 | 0.317 | 0.223 | 0.212 |
| C24:1w9 | | | | |
| | | | | |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 |

Table 16
Fatty Acid Pr file

| | 5538-19A | 5538-19B | LP004-6A | LP004-6B |
|-------------|------------|------------|----------|----------|
| | TRANSGENIC | TRANSGENIC | CONTROL | CONTROL |
| | LRL-2166 | LRL-2167 | LRL-2168 | LRL-2169 |
| C6:0 | 0.004 | 0.005 | | |
| C8:0 | 0.007 | 0.007 | 0.004 | 0.005 |
| C10:0 | 0.012 | 0.012 | 0.008 | 0.008 |
| C12:0 | 0.020 | 0.020 | 0.011 | 0.012 |
| C13:0 | | | | |
| C14:0 | 0.099 | 0.108 | 0.050 | 0.050 |
| C14:1w5 | | | | |
| C15:0 | 0.059 | 0.068 | 0.017 | 0.019 |
| C16:0 | 5.272 | 5.294 | 4.049 | 4.057 |
| C16:1 | 0.350 | 0.417 | 0.197 | 0.208 |
| C16:2 | 0.199 | 0.187 | 0.076 | 0.077 |
| C17:0 | 0.092 | 0.089 | 0.078 | 0.077 |
| C16:3 | 0.149 | 0.149 | 0.192 | 0.198 |
| C16:4 | | 0.010 | | |
| C18:0 | 3.815 | 3.771 | 2.585 | 2.638 |
| C18:1 | 57.562 | 57.051 | 68.506 | 68.352 |
| C18:2 (6,9) | 4.246 | 4.022 | | |
| C18:2w6 | 10.900 | 11.589 | 19.098 | 19.122 |
| C18:2w3 | 0.020 | 0.008 | 0.008 | 0.009 |
| C18:3w6 | 12.565 | 12.595 | 0.013 | 0.015 |
| C18:3w3 | 1.084 | 1.137 | 1.501 | 1.542 |
| C18:4 | 0.017 | 0.013 | 0.011 | 0.008 |
| C18:4 | 0.028 | 0.024 | | |
| C20:0 | 1.138 | 1.104 | 0.937 | 0.943 |
| C20:1 | 1.115 | 1.085 | 1.330 | 1.327 |
| C20:2w6 | 0.150 | 0.143 | 0.068 | 0.071 |
| C20:3w6 | 0.026 | 0.025 | 0.014 | 0.012 |
| C20:4w6 | | | | |
| C20:3w3 | | | | |

Table 16
Fatty Acid Pr file

| | 5538-19A | 5538-19B | LP004-6A | LP004-6B |
|----------------|-------------------|-------------------|-----------------|-----------------|
| | TRANSGENIC | TRANSGENIC | CONTROL | CONTROL |
| | | | | |
| | LRL-2166 | LRL-2167 | LRL-2168 | LRL-2169 |
| | | | | |
| C20:4w3 | | | | |
| C20:5w3 | | | | |
| C22:0 | 0.506 | 0.484 | 0.535 | 0.539 |
| C22:1 | 0.017 | 0.020 | 0.032 | 0.032 |
| C21:5 | | 0.040 | 0.030 | 0.031 |
| C22:4w6 | 0.038 | 0.064 | 0.015 | 0.014 |
| C22:5w6 | | | | |
| C22:5w3 | 0.023 | 0.018 | 0.021 | 0.017 |
| C24:0 | 0.352 | 0.321 | 0.353 | 0.362 |
| C22:6w3 | 0.009 | | | |
| C24:1w9 | 0.129 | 0.121 | 0.260 | 0.255 |
| | | | | |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 |

Example 15

Combined Expression of $\Delta 6$ and $\Delta 12$ Desaturases in *B. napus* Achieved by Crossing

Plants containing either the $\Delta 6$ or the $\Delta 12$ desaturase were crossed and individual F1 half-seeds were analyzed for fatty acid composition by GC. Data from one such cross are given in Table 17. The parents for the cross were 5538-LP004-25-2-25 ($\Delta 6$ expressor) and 5542-SP30021-10-16 ($\Delta 12$ expressor). Reciprocal crosses were made and the results of 25 individual F1 seeds of each are shown in the table. Crosses are described such that the first parent indicated is the female. Both sets of crosses gave approximately the same results. Compared to the parents, the $\Delta^{6,9}$ 18:2 decreased, and the GLA increased. $\Delta^{9,12}$ 18:2 levels are increased in most of the F1's as well. Note that these are F1 seeds and only contain one set of each desaturase. In future generations and selection of events homozygous for each desaturase, the F2 GLA levels obtained may be even higher.

Combining traits by crossing may be preferable to combining traits on one T-DNA in some situations. Particularly if both genes are driven off of the same promoter (in this case napin), issues of promoter silencing may favor this approach over putting multiple cDNAs on one construct.

Alternatively, in some cases, combining multiple cDNAs on one T-DNA may be the method of choice. The results are shown in Table 17.

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|
| 5538-LP004-25-2-25 | 4.23 | 0.13 | 2.4 | 61.78 | 8.77 | 6.34 | 11.58 | 0.92 | 0 | 0 |
| 5542-SP30021-10-16 | 4.09 | 0.1 | 2.03 | 38.4 | 0 | 41.88 | 0 | 11.06 | 0.02 | 0.75 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.9 | 0.04 | 2.31 | 38.58 | 0 | 27.91 | 20.94 | 2.67 | 0.65 | 0.92 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.5 | 0.04 | 1.88 | 36.24 | 0 | 28.68 | 22.54 | 3.36 | 0.85 | 0.78 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.51 | 0.03 | 1.98 | 38.36 | 0 | 29.48 | 19.95 | 3.06 | 0.68 | 0.79 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.95 | 0.04 | 1.86 | 38.65 | 0 | 28.08 | 20.81 | 2.92 | 0.75 | 0.76 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.26 | 0.05 | 2.44 | 40.25 | 0.01 | 28.81 | 18.08 | 2.74 | 0.53 | 0.88 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.13 | 0.04 | 2.33 | 34.48 | 0 | 26.73 | 26.2 | 2.32 | 0.75 | 0.9 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.8 | 0.04 | 2.15 | 38.34 | 0 | 28.95 | 20.64 | 2.63 | 0.65 | 0.81 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.96 | 0.05 | 1.59 | 36.43 | 0 | 29.05 | 21.85 | 3.47 | 0.86 | 0.68 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.04 | 0.04 | 2.5 | 37.75 | 0 | 27.23 | 22.89 | 1.95 | 0.55 | 0.99 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.53 | 0.04 | 1.8 | 34.88 | 0 | 29.17 | 23.42 | 3.42 | 0.9 | 0.74 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.43 | 0.04 | 1.89 | 37.12 | 0 | 29.52 | 20.91 | 3.35 | 0.8 | 0.79 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.58 | 0.03 | 2.55 | 39.54 | 0 | 28.81 | 19.34 | 2.44 | 0.54 | 0.98 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.53 | 0.03 | 2.33 | 39.26 | 0 | 29.07 | 19.5 | 2.61 | 0.59 | 0.91 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.4 | 0.02 | 2.41 | 45.53 | 0 | 28.94 | 13.71 | 2.51 | 0.37 | 0.91 |

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 18:4 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.49 | 0.03 | 2.57 | 40.95 | 0 | 28.52 | 17.97 | 2.63 | 0.58 | 0.99 | 1.43 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.65 | 0.04 | 2.11 | 38.02 | 0 | 29.13 | 20.53 | 2.85 | 0.66 | 0.86 | 1.33 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.97 | 0.03 | 1.99 | 34.95 | 0.01 | 27.15 | 25.71 | 2.38 | 0.79 | 0.81 | 1.38 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.81 | 0.05 | 1.46 | 38.3 | 0 | 31.51 | 17.67 | 3.83 | 0.75 | 0.61 | 1.33 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.98 | 0.05 | 2.03 | 37.14 | 0 | 30.09 | 20.28 | 2.79 | 0.72 | 0.8 | 1.36 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.03 | 0.04 | 2.52 | 42.9 | 0 | 27.79 | 16.66 | 2.64 | 0.54 | 0.9 | 1.29 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.03 | 0.04 | 2.27 | 40.72 | 0 | 29.37 | 17.56 | 2.53 | 0.53 | 0.86 | 1.35 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.98 | 0.04 | 2.61 | 39.91 | 0 | 28.06 | 19.15 | 2.69 | 0.6 | 0.96 | 1.26 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.73 | 0.03 | 1.89 | 40.22 | 0 | 29.44 | 18.21 | 3 | 0.67 | 0.73 | 1.39 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.02 | 0.04 | 2.14 | 42.58 | 0 | 30.36 | 15.18 | 2.43 | 0.42 | 0.82 | 1.3 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.14 | 0.06 | 2.23 | 30.67 | 0 | 30.38 | 25.47 | 3.12 | 0.91 | 0.9 | 1.29 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.05 | 0.07 | 1.7 | 37.03 | 0.04 | 32.1 | 15.97 | 5.38 | 0.96 | 0.69 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.01 | 0.07 | 1.58 | 38.02 | 0.05 | 33.65 | 13.92 | 5.15 | 0.89 | 0.66 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.07 | 0.06 | 2.01 | 31.63 | 0.05 | 31.13 | 23.09 | 3.94 | 1.1 | 0.83 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.03 | 0.05 | 1.94 | 31.88 | 0 | 30.98 | 23.71 | 3.45 | 0.99 | 0.82 | 1.3 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.92 | 0.06 | 1.71 | 35.77 | 0.03 | 33.15 | 16.39 | 5.28 | 0.98 | 0.68 | 1.32 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.09 | 0.08 | 1.57 | 34.6 | 0.03 | 33.73 | 16.73 | 5.48 | 0.99 | 0.66 | 1.28 |

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|-----------|
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.94 | 0.07 | 1.59 | 34.03 | 0.04 | 31.35 | 19.76 | 5.29 | 1.22 | 0.67 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.13 | 0.06 | 1.85 | 31.44 | 0.06 | 31.28 | 23.77 | 3.52 | 1.04 | 0.79 1.22 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.14 | 0.06 | 1.96 | 31.11 | 0.04 | 31.88 | 23.3 | 3.6 | 1.01 | 0.82 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.98 | 0.07 | 1.58 | 35.06 | 0 | 32.06 | 18.1 | 5.33 | 1.12 | 0.67 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.89 | 0.06 | 1.59 | 32.51 | 0.05 | 29.44 | 22.91 | 5.33 | 1.54 | 0.67 1.25 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4 | 0.07 | 1.69 | 32.1 | 0.05 | 30.49 | 22.77 | 4.66 | 1.32 | 0.75 1.26 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.06 | 0.05 | 1.93 | 30.77 | 0.07 | 28.37 | 27.21 | 3.37 | 1.19 | 0.84 1.25 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.1 | 0.06 | 1.9 | 31.77 | 0.05 | 32.33 | 22.03 | 3.92 | 0.98 | 0.78 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.94 | 0.07 | 1.67 | 34.74 | 0.03 | 33.63 | 17.1 | 5.16 | 0.99 | 0.68 1.26 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.71 | 0.06 | 1.65 | 33.05 | 0 | 33.22 | 19.73 | 4.7 | 1.07 | 0.68 1.39 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.84 | 0.06 | 1.71 | 34.16 | 0.04 | 34.52 | 16.74 | 5.18 | 0.97 | 0.68 1.34 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4 | 0.07 | 1.66 | 34.97 | 0.07 | 33.08 | 17.07 | 5.27 | 1.1 | 0.67 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.16 | 0.06 | 1.99 | 35.44 | 0.05 | 31.89 | 18.95 | 3.68 | 0.89 | 0.81 1.29 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.05 | 0.08 | 1.46 | 33.49 | 0 | 31.96 | 18.81 | 6.2 | 1.32 | 0.61 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.2 | 0.06 | 1.93 | 35.06 | 0.06 | 33.69 | 17.38 | 4 | 0.86 | 0.78 1.21 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.07 | 0.06 | 1.74 | 36 | 0.06 | 32.18 | 17.86 | 4.32 | 0.96 | 0.73 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.11 | 0.05 | 2.24 | 29.64 | 0.04 | 28.64 | 27.94 | 3.06 | 1.12 | 0.97 1.26 |

Example 16

Expression of *M. alpina* desaturases in soybean

The *M. alpina* desaturases can be used to drive production of GLA and other PUFAs in soybean by use of the following expression constructs. Two means by which exogenous DNA can be inserted into the soybean genome are *Agrobacterium* infection or particle gun. Particle gun transformation is disclosed in U.S. patent 5,503,998. Plants can be selected using a glyphosate resistance marker (4, 971, 908). *Agrobacterium* transformation of soybean is well established to one of ordinary skill in the art.

For seed specific expression, the coding regions of the desaturase cDNAs are placed under control of the 5' regulatory region of *Glycine max* alpha-type beta conglycinin storage protein gene. The specific region that can be used is nucleotides 78-921 of gi 169928 (Doyle, J.J., Schuler, M.A., Godette, W.D., Zenger, V., Beachy, R.N., and Slightom, J.L., 1986 J. Biol. Chem. 261 (20), 9228-9238). The 3' regulatory region that can be used is from the pea ribulose 1,5 biphosphate carboxylase/oxygenase small subunit (rbcS) gene. The specific sequences to be used are nucleotides 1-645 of gi 169145 (Hunt, A.G. 1988 DNA 7: 329-336).

Since soybean seeds contain more 18:2, and perhaps more endogenous $\Delta 12$ desaturase activity than *Brassica*, the effect of the *Mortierella* $\Delta 12$ desaturase on achieving optimal GLA levels can be tested as follows. A construct containing the $\Delta 6$ cDNA can be used to see if $\Delta^{6,9}$ 18:2 is produced along with GLA. A construct containing the $\Delta 12$ desaturase can be used to see if the amount of 18:2 can be increased in soybean. A construct containing both the $\Delta 6$ and $\Delta 12$ desaturases can be used to produce optimal levels of GLA. Alternatively, plants containing each of the single desaturases may be crossed if necessary to combine the genes.

Similar constructs may be made to express the $\Delta 5$ desaturase alone, or in combination with $\Delta 12$ and/or $\Delta 6$ desaturases.

Example 17

Human Desaturase Gene Sequences

Human desaturase gene sequences potentially involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology
5 between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases were found. As with some other membrane-bound desaturases the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases
10 exhibited homology to *M. alpina* $\Delta 5$, $\Delta 6$, $\Delta 9$, and $\Delta 12$ desaturases.

The *M. alpina* $\Delta 5$ desaturase and $\Delta 6$ desaturase cDNA sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, California 94304. The $\Delta 5$ desaturase sequence was divided into fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-
15 446. The $\Delta 6$ desaturase sequence was divided into three fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames
20 (both strands).

The polypeptide fragments 2 and 3 of *M. alpina* $\Delta 5$ and $\Delta 6$ have homologies with the CloneID sequences as outlined in Table 18. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results have been reviewed, Clone Information was searched with the
25 default settings of Stringency of ≥ 50 , and Productscore ≤ 100 for different CloneID numbers. The Clone Information Results displayed the information including the ClusterID, CloneID, Library, HitID, Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command assembles all of the
30 CloneID which comprise the ClusterID. The following default settings were

used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, Wisconsin 53705) Assembly:

| | | |
|----|-------------------|-----|
| | Word Size: | 7 |
| 5 | Minimum Overlap: | 14 |
| | Stringency: | 0.8 |
| | Minimum Identity: | 14 |
| | Maximum Gap: | 10 |
| | Gap Weight: | 8 |
| 10 | Length Weight: | 2 |

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequences within a contig. The contig containing the CloneID was identified, and the ambiguous sites of the consensus sequence was edited based on the alignment of the CloneIDs (see SEQ ID NO:31 - SEQ ID NO:35) to generate the best possible sequence. The procedure was repeated for all six CloneID listed in Table 18. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan 48105). These consensus sequences were assembled. The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (SEQ ID NO:37). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into protein sequences. The *M. alpina* Δ5 (MA29) and Δ6 (MA524) sequences were compared with each of the translated contigs using the FastA search (a Pearson

and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig. The homology among the *M. alpina* $\Delta 5$ and $\Delta 6$ to contigs 2535 and 3854933 were utilized to create the final contig called 253538a. Figure 9 is the FastA match of the final contig 253538a and MA29, and Figure 10 is the FastA match of the final contig 253538a and MA524. The DNA sequences for the various contigs are presented in SEQ ID NO:31 -SEQ ID NO:37 The various peptide sequences are shown in SEQ ID NO:38 - SEQ ID NO: 44.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acids. It starts with Gln (CAG) and ends with the stop codon (TGA). The contig 253538a aligns with both *M. alpina* $\Delta 5$ and $\Delta 6$ sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Table 18, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

Uses of the Human Desaturases

These human sequences can be expressed in yeast and plants utilizing the procedures described in the preceding examples. For expression in mammalian cells and transgenic animals, these genes may provide superior codon bias. In addition, these sequences can be used to isolate related desaturase genes from other organisms.

Table 18

| Sections of the Desaturases | Clone ID from LifeSeq Database | Keyword |
|-----------------------------|--------------------------------|-----------------------|
| 151-300 $\Delta 5$ | 3808675 | fatty acid desaturase |
| 301-446 $\Delta 5$ | 354535 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 3448789 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 1362863 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 2394760 | $\Delta 6$ |
| 301-457 $\Delta 6$ | 3350263 | $\Delta 6$ |

Example 18**5 Identification of Homologues to *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases**

A nucleic acid sequence that encodes a putative $\Delta 5$ desaturase was identified through a TBLASTN search of the expressed sequence tag databases through NCBI using amino acids 100-446 of Ma29 as a query. The truncated portion of the Ma29 sequence was used to avoid picking up homologies based on the cytochrome b5 portion at the N-terminus of the desaturase. The deduced amino acid sequence of an est from *Dictyostelium discoideum* (accession # C25549) shows very significant homology to Ma29 and lesser, but still significant homology to Ma524. The DNA sequence is presented as SEQ ID NO:45. The amino acid sequence is presented as SEQ ID NO:46.

15

Example 19**Identification of *M. alpina* $\Delta 5$ and $\Delta 6$ homologues in other PUFA-producing organisms**

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Phaeodactylum tricornutum*. A plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative $\Delta 5$ or $\Delta 6$ desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

One clone was identified from the *Phaeodactylum* library with homology to Ma29 and Ma524; it is called 144-011-B12. The DNA sequence is presented as SEQ ID NO:47. The amino acid sequence is presented as SEQ ID NO:48.

5

Example 20

Identification of *M. alpina* $\Delta 5$ and $\Delta 6$ homologues in other PUFA-producing organisms

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Schizochytrium* species. A plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative $\Delta 5$ or $\Delta 6$ desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

15

One clone was identified from the *Schizochytrium* library with homology to Ma29 and Ma524; it is called 81-23-C7. This clone contains a ~1 kb insert. Partial sequence was obtained from each end of the clone using the universal forward and reverse sequencing primers. The DNA sequence from the forward primer is presented as SEQ ID NO:49. The peptide sequence is presented as SEQ ID NO:50. The DNA sequence from the reverse primer is presented as SEQ ID NO:51. The amino acid sequence from the reverse primer is presented as SEQ ID NO:52.

20

Example 21

Nutritional Compositions

25

The PUFAs of the previous examples can be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutrition solutions.

I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron.

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

5 Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity
- Lactose-free formulation to avoid lactose-associated diarrhea
- Low osmolality (240 mOsm/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

20 Ingredients: (Pareve, ®) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, 25 potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic

acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea.

5 Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- 10 • Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- 15 • Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 20 • Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- 25 • Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve, ®) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy

fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono- and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

10 **C. Isomil® SF Sucrose-Free Soy Formula With Iron.**

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- 15 • Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- 20 • Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- 25 • Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve, ©) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1% soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch,

0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride, carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

**D. Isomil® 20 Soy Formula With Iron Ready To Feed,
20 Cal/fl oz.**

Usage: When a soy feeding is desired.

Ingredients: (Pareve, ©) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

E. Similac® Infant Formula

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

Features:

- Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
- 5 • Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
- Carbohydrate as lactose in proportion similar to that of human milk.
- Low renal solute load to minimize stress on developing organs.
- 10 • Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (®-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamid, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, 15 thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron

Usage: For premature infants' special nutritional needs after hospital 20 discharge. Similac NeoCare is a nutritionally complete formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

- 25 • Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 Calories to extend the nutritional support initiated in-hospital.

- More calcium and phosphorus for improved bone mineralization.

Ingredients: ©-D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium-chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: ©-D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono- and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D₃, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art..

II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

Ingredients:

®-D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-

rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:

- 5 • For patients who need extra calories, protein, vitamins and minerals
- Especially useful for people who do not take in enough calories and nutrients
- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

10

Ingredients:

Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein-Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially
15 Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

20

Vitamins and Minerals:

Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta-
25 Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein:

Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

| | | |
|---|---------------------|-----|
| | Soy protein isolate | 74% |
| 5 | Milk proteins | 26% |

Fat:

Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, and corn oils, and soy lecithin.

| | | |
|----|---|-----|
| 10 | Partially hydrogenated cottonseed and soybean oil | 76% |
| | Canola oil | 8% |
| | High-oleic safflower oil | 8% |
| | Corn oil | 4% |
| | Soy lecithin | 4% |

15 **Carbohydrate:**

Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

| | | |
|----|--------------------------|-----|
| | High-fructose corn syrup | 24% |
| 20 | Brown sugar | 21% |
| | Maltodextrin | 12% |
| | Honey | 11% |
| | Crisp rice | 9% |
| | Glycerine | 9% |
| 25 | Soy polysaccharide | 7% |
| | Oat bran | 7%\ |

C. ENSURE® HIGH PROTEIN

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions

- For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features-

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride,

Riboflavin, Folio Acid, Sodium Motybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D.3 and Cyanocobalamin.

Protein:

- 5 The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | |
|-------------------------------|-----|
| Sodium and calcium caseinates | 85% |
| Soy protein isolate | 15% |

Fat:

- 10 The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

| | |
|--------------------------|-----|
| High-oleic safflower oil | 40% |
| Canola oil | 30% |
| Soy oil | 30% |

- 15 The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from
- 20 polyunsaturated fatty acids.

Carbohydrate:

- 25 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORSO® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | |
|---------|-----|
| Sucrose | 60% |
|---------|-----|

| | |
|------------------|-----|
| Maltodextrin | 40% |
| Chocolate | |
| Sucrose | 70% |
| Maltodextrin | 30% |

5

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

10

Patient Conditions:

- For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE
- For healthy adults who don't eat right and need extra nutrition

15

Features:

- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

20

Ingredients:

French Vanilla: ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride),

25

- Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein:

The protein source is calcium caseinate.

| | |
|-------------------|------|
| Calcium caseinate | 100% |
|-------------------|------|

10 Fat

The fat source is a blend of two oils: high-oleic safflower and canola.

| | |
|--------------------------|-----|
| High-oleic safflower oil | 70% |
| Canola oil | 30% |

- The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.

20 Carbohydrate

- ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | |
|--------------|-----|
| Sucrose | 51% |
| Maltodextrin | 49% |

Chocolate

| | |
|--------------|-------|
| Sucrose | 47.0% |
| Corn Syrup | 26.5% |
| Maltodextrin | 26.5% |

5 **Vitamins and Minerals**

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

10

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement.

15 ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:

- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

20

Features

- Rich, creamy taste
- Good source of essential vitamins and minerals

25 **Ingredients**

Vanilla: ®-D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride,

Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D₃.

Protein

The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | |
|-------------------------------|-----|
| Sodium and calcium caseinates | 84% |
| Soy protein isolate | 16% |

Fat

The fat source is corn oil.

| | |
|----------|------|
| Corn oil | 100% |
|----------|------|

Carbohydrate

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, butter pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors

| | |
|--------------|-----|
| Corn Syrup | 39% |
| Maltodextrin | 38% |
| Sucrose | 23% |

Chocolate and eggnog flavors

| | |
|------------|-----|
| Corn Syrup | 36% |
|------------|-----|

Maltodextrin 34%

Sucrose 30%

Vitamins and Minerals

5 An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

10 F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

Patient Conditions:

- For patients with increased calorie and protein needs, such as following surgery or injury
- For patients with limited volume tolerance and early satiety

20 **Features**

- For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaVmL
- High nitrogen
- 25 • Calorically dense

Ingredients

Vanilla: D-Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial
5 Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium
10 Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D₃.

G. ENSURE® POWDER

Usage: ENSURE POWDER (reconstituted with water) is a low-residue
15 liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

- For patients on modified diets
- 20 • For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

Features

- Convenient, easy to mix
- 25 • Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets

- Lactose-free, easily digested

Ingredients: ©-D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate

5 Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide,

10 Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein

The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | | |
|----|-------------------------------|-----|
| | Sodium and calcium caseinates | 84% |
| 15 | Soy protein isolate | 16% |

Fat

The fat source is corn oil.

| | | |
|--|----------|------|
| | Corn oil | 100% |
|--|----------|------|

Carbohydrate

20 ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla

| | | |
|----|--------------|-----|
| 25 | Corn Syrup | 35% |
| | Maltodextrin | 35% |
| | Sucrose | 30% |

H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:

- For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

Features

- Rich and creamy, good taste
- Good source of essential vitamins and minerals Convenient-needs no refrigeration
- Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

Vanilla: ©-D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate. Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein

The protein source is nonfat milk.

Nonfat milk

100%

Fat

The fat source is hydrogenated soybean oil.

| | |
|--------------------------|------|
| Hydrogenated soybean oil | 100% |
|--------------------------|------|

Carbohydrate

- 5 ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors

| | | |
|----|----------------------|-----|
| 10 | Sucrose | 56% |
| | Lactose | 27% |
| | Modified food starch | 17% |

Chocolate

| | | |
|----|----------------------|-----|
| | Sucrose | 58% |
| 15 | Lactose | 26% |
| | Modified food starch | 16% |

I. ENSURE® WITH FIBER

- 20 Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is
- 25 suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions

- For patients who can benefit from increased dietary fiber and nutrients

Features

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- 5 • Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

Ingredients

- 10 **Vanilla:** ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride,
- 15 Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium
- 20 Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein

The protein source is a blend of two high-biologic-value proteins- casein and soy.

| | | |
|----|-------------------------------|-----|
| 25 | Sodium and calcium caseinates | 80% |
| | Soy protein isolate | 20% |

Fat

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

| | | |
|---|--------------------------|-----|
| | High-oleic safflower oil | 40% |
| 5 | Canola oil | 40% |
| | Corn oil | 20% |

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.

Carbohydrate

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | | |
|----|--------------|-----|
| 20 | Maltodextrin | 66% |
| | Sucrose | 25% |
| | Oat Fiber | 7% |
| | Soy Fiber | 2% |

Chocolate

| | | |
|----|--------------|-----|
| 25 | Maltodextrin | 55% |
| | Sucrose | 36% |
| | Oat Fiber | 7% |

Soy Fiber

2%

Fiber

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl-oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs of this invention.

J. Oxepa™ Nutritional Product

Oxepa is low-carbohydrate, calorically dense enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric Distribution:

- Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs.
- The distribution of Calories in Oxepa is shown in Table 7.

| Table 7. Caloric Distribution of Oxepa | | | |
|--|--------------|-----------|----------|
| | per 8 fl oz. | per liter | % of Cal |
| Calories | 355 | 1,500 | --- |
| Fat (g) | 22.2 | 93.7 | 55.2 |
| Carbohydrate (g) | 25 | 105.5 | 28.1 |
| Protein (g) | 14.8 | 62.5 | 16.7 |
| Water (g) | 186 | 785 | --- |

Fat:

- Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).
- The fat source is a oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table 8.

- Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table 10.
- Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

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The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs of this invention.

| Table 8. Typical Fatty Acid Profile | | | |
|-------------------------------------|---------------------|------------|-------|
| | % Total Fatty Acids | g/8 fl oz* | g/L* |
| Caproic (6:0) | 0.2 | 0.04 | 0.18 |
| Caprylic (8:0) | 14.69 | 3.1 | 13.07 |
| Capric (10:0) | 11.06 | 2.33 | 9.87 |
| Palmitic (16:0) | 5.59 | 1.18 | 4.98 |
| Palmitoleic (16:1n-7) | 1.82 | 0.38 | 1.62 |
| Stearic (18:0) | 1.84 | 0.39 | 1.64 |
| Oleic (18:1n-9) | 24.44 | 5.16 | 21.75 |
| Linoleic (18:2n-6) | 16.28 | 3.44 | 14.49 |
| α -Linolenic (18:3n-3) | 3.47 | 0.73 | 3.09 |
| γ -Linolenic (18:3n-6) | 4.82 | 1.02 | 4.29 |
| Eicosapentaenoic (20:5n-3) | 5.11 | 1.08 | 4.55 |
| n-3-Docosapentaenoic (22:5n-3) | 0.55 | 0.12 | 0.49 |
| Docosahexaenoic (22:6n-3) | 2.27 | 0.48 | 2.02 |
| Others | 7.55 | 1.52 | 6.72 |

* Fatty acids equal approximately 95% of total fat.

| Table 9. Fat Profile of Oxepa. | |
|--------------------------------|------------------------------|
| % of total calories from fat | 55.2 |
| Polyunsaturated fatty acids | 31.44 g/L |
| Monounsaturated fatty acids | 25.53 g/L |
| Saturated fatty acids | 32.38 g/L |
| n-6 to n-3 ratio | 1.75:1 |
| Cholesterol | 9.49 mg/8 fl oz 40.1 mg/L |

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Carbohydrate:

- The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).
- The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO₂) production. High CO₂ levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.
- Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

- Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).
- The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.
- Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO₂ production, a high protein diet will increase ventilatory drive.

- The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.
- As demonstrated in Table 11, the amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by theNational Academy of Sciences.
- Oxepa is gluten-free.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

- 10 (i) APPLICANT: KNUTZON, DEBORAH
MURKERJI, PRADIP
HUANG, YUNG-SHENG
THURMOND, JENNIFER
CHAUDHARY, SUNITA
LEONARD, AMANDA
- 15 (ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR SYNTHESIS
OF LONG CHAIN POLY-UNSATURATED FATTY ACIDS IN PLANTS
- (iii) NUMBER OF SEQUENCES: 52
- 20 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LIMBACH & LIMBACH L.L.P.
(B) STREET: 2001 FERRY BUILDING
(C) CITY: SAN FRANCISCO
(D) STATE: CA
25 (E) COUNTRY: USA
(F) ZIP: 94111
- (v) COMPUTER READABLE FORM:
30 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Microsoft Word
- 35 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- 40 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/834,033
(B) FILING DATE: 11-APR-1997
- (vii) PRIOR APPLICATION DATA:
45 (A) APPLICATION NUMBER: US 08/833,610
(B) FILING DATE: 11-APR-1997
- (viii) ATTORNEY/AGENT INFORMATION:
50 (A) NAME: MICHAEL R. WARD
(B) REGISTRATION NUMBER: 38,351
(C) REFERENCE/DOCKET NUMBER: CGAB-320
- (ix) TELECOMMUNICATION INFORMATION:
55 (A) TELEPHONE: (415) 433-4150
(B) TELEFAX: (415) 433-8716
(C) TELEX: N/A

(2) INFORMATION FOR SEQ ID NO:1:

- 60 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1617 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | |
|----|--|------|
| | CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCCTTC AACCCCCCTC TTTGACAAAG | 60 |
| 15 | ACAACAAACC ATGGCTGCTG CTCCCAGTGT GAGGACGTTT ACTCGGGCCG AGGTTTTGAA | 120 |
| | TGCCGAGGCT CTGAATGAGG GCAAGAAGGA TGCCGAGGCA CCCTTCTTGA TGATCATCGA | 180 |
| | CAACAAGGTG TACGATGTCC GCGAGTTCGT CCCTGATCAT CCCGGTGGAA GTGTGATTCT | 240 |
| 20 | CACGCACGTT GGCAAGGACG GCACTGACGT CTTTGACACT TTTCACCCCG AGGCTGCTTG | 300 |
| | GGAGACTCTT GCCAACTTTT ACGTTGGTGA TATTGACGAG AGCGACCGCG ATATCAAGAA | 360 |
| 25 | TGATGACTTT GCGGCCGAGG TCCGCAAGCT GCGTACCTTG TTCCAGTCTC TTGGTTACTA | 420 |
| | CGATTCTTCC AAGGCATACT ACGCCTTCAA GGTCTCGTTC AACCTCTGCA TCTGGGGTTT | 480 |
| | GTCGACGGTC ATTGTGGCCA AGTGGGGCCA GACCTCGACC CTCGCCAACG TGCTCTCGGC | 540 |
| 30 | TGCGCTTTTG GGTCTGTTCT GGCAGCAGTG CGGATGGTTG GCTCAGACT TTTTGCATCA | 600 |
| | CCAGGTCTTC CAGGACCGTT TCTGGGGTGA TCTTTTCGGC GCCTTCTTGG GAGGTGTCTG | 660 |
| 35 | CCAGGGCTTC TCGTCCTCGT GGTGGAAGGA CAAGCACAAC ACTCACCACG CCGCCCCCAA | 720 |
| | CGTCCACGGC GAGGATCCCG ACATTGACAC CCACCTCTG TTGACCTGGA GTGAGCATGC | 780 |
| | GTTGGAGATG TTCTCGGATG TCCCAGATGA GGAGCTGACC CGCATGTGGT CGCGTTTCAT | 840 |
| 40 | GGTCTGAAC CAGACCTGGT TTTACTTCCC CATTCTCTCG TTTGCCCGTC TCTCCTGGTG | 900 |
| | CCTCCAGTCC ATTCTCTTTG TGCTGCCTAA CGGTCAGGCC CACAAGCCCT CGGGCGCGCG | 960 |
| 45 | TGTGCCCATC TCGTTGGTCG AGCAGCTGTC GCTTGCGATG CACTGGACCT GGTACCTCGC | 1020 |
| | CACCATGTTC CTGTTTCATCA AGGATCCCGT CAACATGCTG GTGTACTTTT TGGTGTGCGA | 1080 |
| | GGCGGTGTGC GGAAACTTGT TGGCGATCGT GTTCTCGCTC AACCACAACG GTATGCCTGT | 1140 |
| 50 | GATCTCGAAG GAGGAGGCGG TCGATATGGA TTTCTTCACG AAGCAGATCA TCACGGGTCTG | 1200 |
| | TGATGTCCAC CCGGGTCTAT TTGCCAACTG GTTCACGGGT GGATTGAACT ATCAGATCGA | 1260 |
| 55 | GCACCACTTG TTCCCTTCGA TGCCTCGCCA CAACTTTTCA AAGATCCAGC CTGCTGTGCGA | 1320 |
| | GACCCTGTGC AAAAAGTACA ATGTCCGATA CCACACCACC GGTATGATCG AGGGAACATGC | 1380 |
| | AGAGGTCTTT AGCCGTCTGA ACGAGGTCTC CAAGGCTGCC TCCAAGATGG GTAAGGCGCA | 1440 |
| 60 | GTAACAAAAA AAACAAGGAC GTTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT | 1500 |
| | TGTCAAGTCG AGCGTTTCTG GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC | 1560 |

CCCCCGCTCA TATCTCATTC ATTTCTCTTA TTAAACAACT TGTTCACCCG TTCACCG 1617

5

(2) INFORMATION FOR SEQ ID NO:2:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 457 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25

Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
1 5 10 15

30

Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
20 25 30

35

Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
35 40 45

Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
50 55 60

Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
65 70 75 80

Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
85 90 95

40

Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
100 105 110

Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
115 120 125

45

Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
130 135 140

50

Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
145 150 155 160

Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
165 170 175

55

His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
180 185 190

Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
195 200 205

60

His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
210 215 220

Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
 225 230 235 240
 5 Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
 245 250 255
 Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
 260 265 270
 10 Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
 275 280 285
 Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
 290 295 300
 15 Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
 305 310 315 320
 20 Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
 325 330 335
 Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
 340 345 350
 25 Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
 355 360 365
 Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
 370 375 380
 30 Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
 385 390 395 400
 35 Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
 405 410 415
 Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
 420 425 430
 40 Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
 435 440 445
 Ala Ala Ser Lys Met Gly Lys Ala Gln
 450 455

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1488 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCCCTGTC GCTGTCGGCA CACCCCATCC TCCCTCGCTC CCTCTGCGTT TGTCTTGGC 60

5 CCACCGTCTC TCCTCCACCC TCCGAGACGA CTGCAACTGT AATCAGGAAC CGACAAATAC 120
 ACGATTTCTT TTTACTCAGC ACCAACTCAA AATCCTCAAC CGCAACCCTT TTTCAGGATG 180
 10 GCACCTCCCA ACACTATCGA TGCCGGTTTG ACCCAGCGTC ATATCAGCAC CTCGGCCCCA 240
 AACTCGGCCA AGCCTGCCTT CGAGCGCAAC TACCAGCTCC CCGAGTTCAC CATCAAGGAG 300
 ATCCGAGAGT GCATCCCTGC CCACTGCTTT GAGCGCTCCG GTCTCCGTGG TCTCTGCCAC 360
 GTTGCCATCG ATCTGACTTG GCGCTCGCTC TTGTTCTGG CTGCGACCCA GATCGACAAG 420
 TTTGAGAATC CCTTGATCCG CTATTTGGCC TGGCCTGTTT ACTGGATCAT GCAGGGTATT 480
 15 GTCTGCACCG GTGTCTGGGT GCTGGCTCAC GAGTGTGGTC ATCAGTCCTT CTCGACCTCC 540
 AAGACCCTCA ACAACACAGT TGGTTGGATC TTGCACTCGA TGCTCTTGGT CCCCTACCAC 600
 20 TCCTGGAGAA TCTCGCACTC GAAGCACCAC AAGGCCACTG GCCATATGAC CAAGGACCAG 660
 GTCTTTGTGC CCAAGACCCG CTCCCAGGTT GGCTTGCCCTC CCAAGGAGAA CGCTGCTGCT 720
 GCCGTTCAGG AGGAGGACAT GTCCGTGCAC CTGGATGAGG AGGCTCCCAT TGTGACTTTG 780
 25 TTCTGGATGG TGATCCAGTT CTTGTTCCGA TGGCCCGCGT ACCTGATTAT GAACGCCTCT 840
 GGCCAAGACT ACGGCCGCTG GACCTCGCAC TTCCACACGT ACTCGCCCAT CTTTGAGCCC 900
 30 CGCAACTTTT TCGACATTAT TATCTCGGAC CTCGGTGTGT TGGCTGCCCT CGGTGCCCTG 960
 ATCTATGCCT CCATGCAGTT GTCGCTCTTG ACCGTACCA AGTACTATAT TGTCCCCTAC 1020
 CTCTTTGTCA ACTTTTGGTT GTCCTGATC ACCTTCTTGC AGCACACCGA TCCCAAGCTG 1080
 35 CCCCATTACC GCGAGGGTGC CTGGAATTC CAGCGTGGAG CTCTTTGCAC CGTTGACCGC 1140
 TCGTTTGGCA AGTTCTTGGA CCATATGTTT CACGGCATTG TCCACACCCA TGTGGCCCAT 1200
 40 CACTTGTTCT CGCAAATGCC GTTCTACCAT GCTGAGGAAG CTACCTATCA TCTCAAGAAA 1260
 CTGCTGGGAG AGTACTATGT GTACGACCCA TCCCCGATCG TCGTTGCGGT CTGGAGGTCG 1320
 TTCCGTGAGT GCCGATTCGT GGAGGATCAG GGAGACGTGG TCTTTTCAA GAAGTAAAAA 1380
 45 AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC 1440
 CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTCATTC GCGCCTCC 1488

50 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

55 (C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Met | Ala | Pro | Pro | Asn | Thr | Ile | Asp | Ala | Gly | Leu | Thr | Gln | Arg | His | Ile | |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| 5 | Ser | Thr | Ser | Ala | Pro | Asn | Ser | Ala | Lys | Pro | Ala | Phe | Glu | Arg | Asn | Tyr | |
| | | | | 20 | | | | | 25 | | | | | 30 | | | |
| | Gln | Leu | Pro | Glu | Phe | Thr | Ile | Lys | Glu | Ile | Arg | Glu | Cys | Ile | Pro | Ala | |
| 10 | | | 35 | | | | | 40 | | | | | 45 | | | | |
| | His | Cys | Phe | Glu | Arg | Ser | Gly | Leu | Arg | Gly | Leu | Cys | His | Val | Ala | Ile | |
| | | 50 | | | | | 55 | | | | | 60 | | | | | |
| 15 | Asp | Leu | Thr | Trp | Ala | Ser | Leu | Leu | Phe | Leu | Ala | Ala | Thr | Gln | Ile | Asp | |
| | 65 | | | | | 70 | | | | | 75 | | | | | 80 | |
| | Lys | Phe | Glu | Asn | Pro | Leu | Ile | Arg | Tyr | Leu | Ala | Trp | Pro | Val | Tyr | Trp | |
| | | | | 85 | | | | | | 90 | | | | | 95 | | |
| 20 | Ile | Met | Gln | Gly | Ile | Val | Cys | Thr | Gly | Val | Trp | Val | Leu | Ala | His | Glu | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | |
| | Cys | Gly | His | Gln | Ser | Phe | Ser | Thr | Ser | Lys | Thr | Leu | Asn | Asn | Thr | Val | |
| 25 | | | 115 | | | | | 120 | | | | | 125 | | | | |
| | Gly | Trp | Ile | Leu | His | Ser | Met | Leu | Leu | Val | Pro | Tyr | His | Ser | Trp | Arg | |
| | | 130 | | | | | 135 | | | | | 140 | | | | | |
| 30 | Ile | Ser | His | Ser | Lys | His | His | Lys | Ala | Thr | Gly | His | Met | Thr | Lys | Asp | |
| | 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| | Gln | Val | Phe | Val | Pro | Lys | Thr | Arg | Ser | Gln | Val | Gly | Leu | Pro | Pro | Lys | |
| | | | | | 165 | | | | | 170 | | | | | 175 | | |
| 35 | Glu | Asn | Ala | Ala | Ala | Ala | Val | Gln | Glu | Glu | Asp | Met | Ser | Val | His | Leu | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| | Asp | Glu | Glu | Ala | Pro | Ile | Val | Thr | Leu | Phe | Trp | Met | Val | Ile | Gln | Phe | |
| 40 | | | 195 | | | | 200 | | | | | | 205 | | | | |
| | Leu | Phe | Gly | Trp | Pro | Ala | Tyr | Leu | Ile | Met | Asn | Ala | Ser | Gly | Gln | Asp | |
| | | 210 | | | | | 215 | | | | | 220 | | | | | |
| 45 | Tyr | Gly | Arg | Trp | Thr | Ser | His | Phe | His | Thr | Tyr | Ser | Pro | Ile | Phe | Glu | |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| | Pro | Arg | Asn | Phe | Phe | Asp | Ile | Ile | Ile | Ser | Asp | Leu | Gly | Val | Leu | Ala | |
| | | | | 245 | | | | | | 250 | | | | | 255 | | |
| 50 | Ala | Leu | Gly | Ala | Leu | Ile | Tyr | Ala | Ser | Met | Gln | Leu | Ser | Leu | Leu | Thr | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| | Val | Thr | Lys | Tyr | Tyr | Ile | Val | Pro | Tyr | Leu | Phe | Val | Asn | Phe | Trp | Leu | |
| 55 | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Val | Leu | Ile | Thr | Phe | Leu | Gln | His | Thr | Asp | Pro | Lys | Leu | Pro | His | Tyr | |
| | | 290 | | | | | 295 | | | | | 300 | | | | | |
| 60 | Arg | Glu | Gly | Ala | Trp | Asn | Phe | Gln | Arg | Gly | Ala | Leu | Cys | Thr | Val | Asp | |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 | |

5

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35

GTATCGCCTG ATTGTTCCCC TGCAGTATCT GCCCCTGGGC AAGGTGCTGC TCTTGTTTAC 1020
 GGTGCGGGAC ATGGTGTCTG CTTACTGGCT GGCCTGACC TTCCAGGCGA ACCACGTTGT 1080
 5 TGAGGAAGTT CAGTGGCCGT TGCCTGACGA GAACGGGATC ATCCAAAAGG ACTGGGCAGC 1140
 TATGCAGGTC GAGACTACGC AGGATTACGC ACACGATTGC CACCTCTGGA CCAGCATCAC 1200
 10 TGGCAGCTTG AACTACCAGG CTGTGCACCA TCTGTTCCCC AACGTGTCGC AGCACCATTA 1260
 TCCCGATATT CTGGCCATCA TCAAGAACAC CTGCAGCGAG TACAAGGTTC CATACTTGT 1320
 CAAGGATACG TTTTGGCAAG CATTTGCTTC ACATTTGGAG CACTTGCGTG TTCTTGGACT 1380
 15 CCGTCCCAAG GAAGAGTAGA AGAAAAAAG CGCCGAATGA AGTATTGCCC CCTTTTCTC 1440
 CAAGAATGGC AAAAGGAGAT CAAGTGGACA TTCTCTATGA AGA 1483

20 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 446 amino acids
 (B) TYPE: amino acid
 25 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35 Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala
 1 5 10 15
 His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr
 20 25 30
 40 Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu
 35 40 45
 Leu Leu Gly Ala Gly Arg Asp Val Thr Pro Val Phe Glu Met Tyr His
 50 55 60
 Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr
 65 70 75 80
 50 Leu Val Ser Asn Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His
 85 90 95
 Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile
 100 105 110
 55 Asp Pro Lys Asn Arg Pro Glu Ile Trp Gly Arg Tyr Ala Leu Ile Phe
 115 120 125
 Gly Ser Leu Ile Ala Ser Tyr Tyr Ala Gln Leu Phe Val Pro Phe Val
 130 135 140
 60 Val Glu Arg Thr Trp Leu Gln Val Val Phe Ala Ile Ile Met Gly Phe
 145 150 155 160

5 Ala Cys Ala Gln Val Gly Leu Asn Pro Leu His Asp Ala Ser His Phe
 165 170 175
 10 Ser Val Thr His Asn Pro Thr Val Trp Lys Ile Leu Gly Ala Thr His
 180 185 190
 15 Asp Phe Phe Asn Gly Ala Ser Tyr Leu Val Trp Met Tyr Gln His Met
 195 200 205
 20 Leu Gly His His Pro Tyr Thr Asn Ile Ala Gly Ala Asp Pro Asp Val
 210 215 220
 25 Ser Thr Ser Glu Pro Asp Val Arg Arg Ile Lys Pro Asn Gln Lys Trp
 225 230 235 240
 30 Phe Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly
 245 250 255
 35 Leu Leu Ala Phe Lys Val Arg Ile Gln Asp Ile Asn Ile Leu Tyr Phe
 260 265 270
 40 Val Lys Thr Asn Asp Ala Ile Arg Val Asn Pro Ile Ser Thr Trp His
 275 280 285
 45 Thr Val Met Phe Trp Gly Gly Lys Ala Phe Phe Val Trp Tyr Arg Leu
 290 295 300
 50 Ile Val Pro Leu Gln Tyr Leu Pro Leu Gly Lys Val Leu Leu Leu Phe
 305 310 315 320
 55 Thr Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln
 325 330 335
 60 Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn
 340 345 350
 65 Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln
 355 360 365
 70 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu
 370 375 380
 75 Asn Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His
 385 390 395 400
 80 Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys
 405 410 415
 85 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His
 420 425 430
 90 Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu
 435 440 445

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 355 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10

Glu Val Arg Lys Leu Arg Thr Leu Phe Gln Ser Leu Gly Tyr Tyr Asp
1 5 10 15

Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val Ser Phe Asn Leu Cys Ile
20 25 30

15

Trp Gly Leu Ser Thr Val Ile Val Ala Lys Trp Gly Gln Thr Ser Thr
35 40 45

20

Leu Ala Asn Val Leu Ser Ala Ala Leu Leu Gly Leu Phe Trp Gln Gln
50 55 60

Cys Gly Trp Leu Ala His Asp Phe Leu His His Gln Val Phe Gln Asp
65 70 75 80

25

Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe Leu Gly Gly Val Cys Gln
85 90 95

Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys His Asn Thr His His Ala
100 105 110

30

Ala Pro Asn Val His Gly Glu Asp Pro Asp Ile Asp Thr His Pro Leu
115 120 125

Leu Thr Trp Ser Glu His Ala Leu Glu Met Phe Ser Asp Val Pro Asp
130 135 140

35

Glu Glu Leu Thr Arg Met Trp Ser Arg Phe Met Val Leu Asn Gln Thr
145 150 155 160

40

Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala Arg Leu Ser Trp Cys Leu
165 170 175

Gln Ser Ile Leu Phe Val Leu Pro Asn Gly Gln Ala His Lys Pro Ser
180 185 190

45

Gly Ala Arg Val Pro Ile Ser Leu Val Glu Gln Leu Ser Leu Ala Met
195 200 205

His Trp Thr Trp Tyr Leu Ala Thr Met Phe Leu Phe Ile Lys Asp Pro
210 215 220

50

Val Asn Met Leu Val Tyr Phe Leu Val Ser Gln Ala Val Cys Gly Asn
225 230 235 240

Leu Leu Ala Ile Val Phe Ser Leu Asn His Asn Gly Met Pro Val Ile
245 250 255

55

Ser Lys Glu Glu Ala Val Asp Met Asp Phe Phe Thr Lys Gln Ile Ile
260 265 270

60

Thr Gly Arg Asp Val His Pro Gly Leu Phe Ala Asn Trp Phe Thr Gly
275 280 285

Gly Leu Asn Tyr Gln Ile Glu His His Leu Phe Pro Ser Met Pro Arg
 290 295 300
 5 His Asn Phe Ser Lys Ile Gln Pro Ala Val Glu Thr Leu Cys Lys Lys
 305 310 315 320
 Tyr Asn Val Arg Tyr His Thr Thr Gly Met Ile Glu Gly Thr Ala Glu
 325 330 335
 10 Val Phe Ser Arg Leu Asn Glu Val Ser Lys Ala Ala Ser Lys Met Gly
 340 345 350
 Lys Ala Gln
 15 355

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 104 amino acids
 20 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Thr Leu Tyr Thr Leu Ala Phe Val Ala Ala Asn Ser Leu Gly Val
 1 5 10 15
 35 Leu Tyr Gly Val Leu Ala Cys Pro Ser Val Xaa Pro His Gln Ile Ala
 20 25 30
 Ala Gly Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile Gly Xaa
 35 40 45
 40 Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Asn Asn Xaa Phe
 50 55 60
 45 Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ile Ala Trp Trp
 65 70 75 80
 Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser Leu Asp Tyr
 85 90 95
 50 Gly Pro Asn Leu Gln His Ile Pro
 100

(2) INFORMATION FOR SEQ ID NO:9:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 252 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

5 Gly Val Leu Tyr Gly Val Leu Ala Cys Thr Ser Val Phe Ala His Gln
1 5 10 15

Ile Ala Ala Ala Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile
20 25 30

10 Gly His Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Tyr Asn
35 40 45

15 Arg Phe Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ser Ile
50 55 60

Ala Trp Trp Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser
65 70 75 80

20 Leu Asp Tyr Asp Pro Asp Leu Gln His Ile Pro Val Phe Ala Val Ser
85 90 95

25 Thr Lys Phe Phe Ser Ser Leu Thr Ser Arg Phe Tyr Asp Arg Lys Leu
100 105 110

Thr Phe Gly Pro Val Ala Arg Phe Leu Val Ser Tyr Gln His Phe Thr
115 120 125

30 Tyr Tyr Pro Val Asn Cys Phe Gly Arg Ile Asn Leu Phe Ile Gln Thr
130 135 140

Phe Leu Leu Leu Phe Ser Lys Arg Glu Val Pro Asp Arg Ala Leu Asn
145 150 155 160

35 Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro Leu Leu Val Ser
165 170 175

Cys Leu Pro Asn Trp Pro Glu Arg Phe Phe Phe Val Phe Thr Ser Phe
180 185 190

40 Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu Asn His Phe Ala
195 200 205

45 Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp Trp Phe Glu Lys
210 215 220

Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser Tyr Met Asp Trp
225 230 235 240

50 Phe Phe Gly Gly Leu Gln Phe Gln Leu Glu His His
245 250

(2) INFORMATION FOR SEQ ID NO:10:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Xaa Xaa Asn Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro
 1 5 10 15

10 Leu Leu Val Ser Cys Leu Pro Asn Trp Pro Glu Arg Phe Xaa Phe Val
 20 25 30

Phe Thr Gly Phe Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu
 35 40 45

15 Asn His Phe Ala Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp
 50 55 60

Trp Phe Glu Lys Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser
 65 70 75 80

20 Tyr Met Asp Trp Phe Phe Cys Gly Leu Gln Phe Gln Leu Glu His His
 85 90 95

25 Leu Phe Pro Arg Leu Pro Arg Cys His Leu Arg Lys Val Ser Pro Val
 100 105 110

Gly Gln Arg Gly Phe Gln Arg Lys Xaa Asn Leu Ser Xaa
 115 120 125

30 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

35 (B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Ala Thr Glu Val Gly Gly Leu Ala Trp Met Ile Thr Phe Tyr Val
 1 5 10 15

Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys Ala Phe Leu
 20 25 30

50 Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp Phe Val Trp
 35 40 45

55 Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His Asp Arg Asn
 50 55 60

Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn Val His Lys
 65 70 75 80

60 Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile Glu
 85 90 95

His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His Xaa Val Ala
 100 105 110

5 Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser
 115 120 125

Lys Pro Leu
 130

10 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 87 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

25 Cys Ser Pro Lys Ser Ser Pro Thr Arg Asn Met Thr Pro Ser Pro Phe
 1 5 10 15

Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
 20 25 30

Phe Pro Thr Met Pro Arg Cys Asn Leu Asn Arg Cys Met Lys Tyr Val
 35 40 45

35 Lys Glu Trp Cys Ala Glu Asn Asn Leu Pro Tyr Leu Val Asp Asp Tyr
 50 55 60

Phe Val Gly Tyr Asn Leu Asn Leu Gln Gln Leu Lys Asn Met Ala Glu
 65 70 75 80

40 Leu Val Gln Ala Lys Ala Ala
 85

(2) INFORMATION FOR SEQ ID NO:13:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: peptide

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

60 Arg His Glu Ala Ala Arg Gly Gly Thr Arg Leu Ala Tyr Met Leu Val
 1 5 10 15

Cys Met Gln Trp Thr Asp Leu Leu Trp Ala Ala Ser Phe Tyr Ser Arg
 20 25 30

(2) INFORMATION FOR SEQ ID NO:14:

(ii) MOLECULE TYPE: peptide

-147-

Asn Tyr Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn Gly
 130 135 140
 5 Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln Asp
 145 150 155 160
 Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu Asn
 165 170 175
 10 Tyr Gln Xaa Val His His Leu Phe Pro His
 180 185

(2) INFORMATION FOR SEQ ID NO:15:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear
 20 (ii) MOLECULE TYPE: peptide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Xaa Xaa His His
 1 5

(2) INFORMATION FOR SEQ ID NO:16:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 446 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear
 40 (ii) MOLECULE TYPE: peptide

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn
 1 5 10 15
 50 His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr
 20 25 30
 Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu
 35 40 45
 55 Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
 50 55 60
 Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr
 65 70 75 80
 60 Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Val Tyr Arg Lys Leu
 85 90 95

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Val | Phe | Glu | Phe | Ser | Lys | Met | Gly | Leu | Tyr | Asp | Lys | Lys | Gly | His | Ile | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | |
| 5 | Met | Phe | Ala | Thr | Leu | Cys | Phe | Ile | Ala | Met | Leu | Phe | Ala | Met | Ser | Val | |
| | | | 115 | | | | | 120 | | | | | 125 | | | | |
| | Tyr | Gly | Val | Leu | Phe | Cys | Glu | Gly | Val | Leu | Val | His | Leu | Phe | Ser | Gly | |
| 10 | | 130 | | | | | 135 | | | | | 140 | | | | | |
| | Cys | Leu | Met | Gly | Phe | Leu | Trp | Ile | Gln | Ser | Gly | Trp | Ile | Gly | His | Asp | |
| | 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| | Ala | Gly | His | Tyr | Met | Val | Val | Ser | Asp | Ser | Arg | Leu | Asn | Lys | Phe | Met | |
| 15 | | | | | 165 | | | | | 170 | | | | | 175 | | |
| | Gly | Ile | Phe | Ala | Ala | Asn | Cys | Leu | Ser | Gly | Ile | Ser | Ile | Gly | Trp | Trp | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| 20 | Lys | Trp | Asn | His | Asn | Ala | His | His | Ile | Ala | Cys | Asn | Ser | Leu | Glu | Tyr | |
| | | | 195 | | | | | 200 | | | | | 205 | | | | |
| | Asp | Pro | Asp | Leu | Gln | Tyr | Ile | Pro | Phe | Leu | Val | Val | Ser | Ser | Lys | Phe | |
| 25 | | 210 | | | | | 215 | | | | | 220 | | | | | |
| | Phe | Gly | Ser | Leu | Thr | Ser | His | Phe | Tyr | Glu | Lys | Arg | Leu | Thr | Phe | Asp | |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| | Ser | Leu | Ser | Arg | Phe | Phe | Val | Ser | Tyr | Gln | His | Trp | Thr | Phe | Tyr | Pro | |
| 30 | | | | | 245 | | | | | 250 | | | | | 255 | | |
| | Ile | Met | Cys | Ala | Ala | Arg | Leu | Asn | Met | Tyr | Val | Gln | Ser | Leu | Ile | Met | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| 35 | Leu | Leu | Thr | Lys | Arg | Asn | Val | Ser | Tyr | Arg | Ala | Gln | Glu | Leu | Leu | Gly | |
| | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Cys | Leu | Val | Phe | Ser | Ile | Trp | Tyr | Pro | Leu | Leu | Val | Ser | Cys | Leu | Pro | |
| 40 | | 290 | | | | | 295 | | | | | 300 | | | | | |
| | Asn | Trp | Gly | Glu | Arg | Ile | Met | Phe | Val | Ile | Ala | Ser | Leu | Ser | Val | Thr | |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 | |
| | Gly | Met | Gln | Gln | Val | Gln | Phe | Ser | Leu | Asn | His | Phe | Ser | Ser | Ser | Val | |
| 45 | | | | | 325 | | | | | 330 | | | | | | 335 | |
| | Tyr | Val | Gly | Lys | Pro | Lys | Gly | Asn | Asn | Trp | Phe | Glu | Lys | Gln | Thr | Asp | |
| | | | | 340 | | | | | 345 | | | | | 350 | | | |
| 50 | Gly | Thr | Leu | Asp | Ile | Ser | Cys | Pro | Pro | Trp | Met | Asp | Trp | Phe | His | Gly | |
| | | | 355 | | | | | 360 | | | | | 365 | | | | |
| | Gly | Leu | Gln | Phe | Gln | Ile | Glu | His | His | Leu | Phe | Pro | Lys | Met | Pro | Arg | |
| 55 | | | | | | | 375 | | | | | 380 | | | | | |
| | Cys | Asn | Leu | Arg | Lys | Ile | Ser | Pro | Tyr | Val | Ile | Glu | Leu | Cys | Lys | Lys | |
| | 385 | | | | | 390 | | | | | 395 | | | | | 400 | |
| | His | Asn | Leu | Pro | Tyr | Asn | Tyr | Ala | Ser | Phe | Ser | Lys | Ala | Asn | Glu | Met | |
| 60 | | | | | 405 | | | | | 410 | | | | | 415 | | |

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
 420 425 430

5 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr
 435 440 445

(2) INFORMATION FOR SEQ ID NO:17:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 359 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
 1 5 10 15
 25 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
 20 25 30
 30 Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
 35 40 45
 Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
 50 55 60
 35 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
 65 70 75 80
 Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
 85 90 95
 40 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
 100 105 110
 45 Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
 115 120 125
 Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
 130 135 140
 50 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
 145 150 155 160
 Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
 165 170 175
 55 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
 180 185 190
 His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
 195 200 205
 60 Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
 210 215 220

5 Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
 225 230 235 240
 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
 245 250 255
 10 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
 260 265 270
 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
 275 280 285
 15 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
 290 295 300
 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
 305 310 315 320
 20 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
 325 330 335
 Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
 340 345 350
 25 Glu Ala Met Gly Lys Ala Ser
 355

(2) INFORMATION FOR SEQ ID NO:18:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 365 amino acids
 (B) TYPE: amino acid
 35 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

45 Met Thr Ser Thr Thr Ser Lys Val Thr Phe Gly Lys Ser Ile Gly Phe
 1 5 10 15
 Arg Lys Glu Leu Asn Arg Arg Val Asn Ala Tyr Leu Glu Ala Glu Asn
 20 25 30
 50 Ile Ser Pro Arg Asp Asn Pro Pro Met Tyr Leu Lys Thr Ala Ile Ile
 35 40 45
 Leu Ala Trp Val Val Ser Ala Trp Thr Phe Val Val Phe Gly Pro Asp
 50 55 60
 55 Val Leu Trp Met Lys Leu Leu Gly Cys Ile Val Leu Gly Phe Gly Val
 65 70 75 80
 60 Ser Ala Val Gly Phe Asn Ile Ser His Asp Gly Asn His Gly Gly Tyr
 85 90 95

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Ser | Lys | Tyr | Gln | Trp | Val | Asn | Tyr | Leu | Ser | Gly | Leu | Thr | His | Asp | Ala | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | |
| 5 | Ile | Gly | Val | Ser | Ser | Tyr | Leu | Trp | Lys | Phe | Arg | His | Asn | Val | Leu | His | |
| | | | 115 | | | | | 120 | | | | | 125 | | | | |
| | His | Thr | Tyr | Thr | Asn | Ile | Leu | Gly | His | Asp | Val | Glu | Ile | His | Gly | Asp | |
| | | | 130 | | | | 135 | | | | | 140 | | | | | |
| 10 | Glu | Leu | Val | Arg | Met | Ser | Pro | Ser | Met | Glu | Tyr | Arg | Trp | Tyr | His | Arg | |
| | | | | | | 150 | | | | | 155 | | | | | 160 | |
| | Tyr | Gln | His | Trp | Phe | Ile | Trp | Phe | Val | Tyr | Pro | Phe | Ile | Pro | Tyr | Tyr | |
| 15 | | | | | 165 | | | | | 170 | | | | | 175 | | |
| | Trp | Ser | Ile | Ala | Asp | Val | Gln | Thr | Met | Leu | Phe | Lys | Arg | Gln | Tyr | His | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| 20 | Asp | His | Glu | Ile | Pro | Ser | Pro | Thr | Trp | Val | Asp | Ile | Ala | Thr | Leu | Leu | |
| | | | 195 | | | | | 200 | | | | | 205 | | | | |
| | Ala | Phe | Lys | Ala | Phe | Gly | Val | Ala | Val | Phe | Leu | Ile | Ile | Pro | Ile | Ala | |
| | | | 210 | | | | 215 | | | | | 220 | | | | | |
| 25 | Val | Gly | Tyr | Ser | Pro | Leu | Glu | Ala | Val | Ile | Gly | Ala | Ser | Ile | Val | Tyr | |
| | | | | | | 230 | | | | | 235 | | | | | 240 | |
| | Met | Thr | His | Gly | Leu | Val | Ala | Cys | Val | Val | Phe | Met | Leu | Ala | His | Val | |
| 30 | | | | | 245 | | | | | 250 | | | | | 255 | | |
| | Ile | Glu | Pro | Ala | Glu | Phe | Leu | Asp | Pro | Asp | Asn | Leu | His | Ile | Asp | Asp | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| 35 | Glu | Trp | Ala | Ile | Ala | Gln | Val | Lys | Thr | Thr | Val | Asp | Phe | Ala | Pro | Asn | |
| | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Asn | Thr | Ile | Ile | Asn | Trp | Tyr | Val | Gly | Gly | Leu | Asn | Tyr | Gln | Thr | Val | |
| | | | | | | | 295 | | | | | 300 | | | | | |
| 40 | His | His | Leu | Phe | Pro | His | Ile | Cys | His | Ile | His | Tyr | Pro | Lys | Ile | Ala | |
| | | | | | | 310 | | | | | 315 | | | | | 320 | |
| | Pro | Ile | Leu | Ala | Glu | Val | Cys | Glu | Glu | Phe | Gly | Val | Asn | Tyr | Ala | Val | |
| 45 | | | | | 325 | | | | | 330 | | | | | 335 | | |
| | His | Gln | Thr | Phe | Phe | Gly | Ala | Leu | Ala | Ala | Asn | Tyr | Ser | Trp | Leu | Lys | |
| | | | | 340 | | | | 345 | | | | | | 350 | | | |
| 50 | Lys | Met | Ser | Ile | Asn | Pro | Glu | Thr | Lys | Ala | Ile | Glu | Gln | | | | |
| | | | 355 | | | | | 360 | | | | | 365 | | | | |

(2) INFORMATION FOR SEQ ID NO:19:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 CCAAGCTTCT GCAGGAGCTC TTTTTTTTTT TTTT 35

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

20 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 21
(D) OTHER INFORMATION: /number= 1
/note= "N=Inosine or Cytosine"

25 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 27
(D) OTHER INFORMATION: /number= 2
/note= "N=Inosine or Cytosine"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 CUACUACUAC UACAYCAYAC NTAYACNAAY AT 32

(2) INFORMATION FOR SEQ ID NO:21:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

50 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 13
(D) OTHER INFORMATION: /number= 1
/note= "N=Inosine or Cytosine"

55 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 19
(D) OTHER INFORMATION: /number= 2
/note= "N=Inosine or Cytosine"

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAUCAUCAUC AUNGGRAANA RRTGRTG 27

(2) INFORMATION FOR SEQ ID NO:22:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
CUACUACUAC UAGGAGTCCT CTACGGTGTT TTG 33

20 (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
35 CAUCAUCAUC AUATGATGCT CAAGCTGAAA CTG 33

(2) INFORMATION FOR SEQ ID NO:24:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
Gln Xaa Xaa His His
1 5

55 (2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
60 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CUACUACUAC UACTCGAGCA AGATGGGAAC GGACCAAGG

39

10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

25

CAUCAUCAUC AUCTCGAGCT ACTCTTCCTT GGGACGGAG

39

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CUACUACUAC UATCTAGACT CGAGACCATG GCTGCTGCTC CAGTGTG

47

45

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

50

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CAUCAUCAUC AUAGGCCTCG AGTTACTGCG CCTTACCCAT

60

40

(2) INFORMATION FOR SEQ ID NO:29:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

15 CUACUACUA CUAGGATCCA TGGCACCTCC CAACACT

37

(2) INFORMATION FOR SEQ ID NO:30:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 42 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

30 CAUCAUCAU CAUGGTACCT CGAGTTACTT CTTGAAAAAG AC

42

(2) INFORMATION FOR SEQ ID NO:31:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1219 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2692004)

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCACGCCGAC CGGCGCCGGG AGATCCTGGC AAAGTATCCA GAGATAAAGT CCTTGATGAA 60
 50 ACCTGATCCC AATTTGATAT GGATTATAAT TATGATGGTT CTCACCCAGT TGGGTGCATT 120
 TTACATAGTA AAAGACTTGG ACTGGAAATG GGCATATTTT GGGGCCTATG CGTTTGGCAG 180
 55 TTGCATTAAC CACTCAATGA CTCTGGCTAT TCATGAGATT GCCACAATG CTGCCTTTGG 240
 CAACTGCAAA GCAATGTGGA ATCGCTGGTT TGGAATGTTT GCTAATCTTC CTATTGGGAT 300
 TCCATATTCA ATTTCTTTA AGAGGTATCA CATGGATCAT CATCGGTACC TTGGAGCTGA 360
 60 TGGCGTCGAT GTAGATATTC CTACCGATTT TGAGGGCTGG TTCTTCTGTA CCGCTTTCAG 420
 AAAGTTTATA TGGGTTATTC TTCAGCCTCT CTTTTATGCC TTTCGACCTC TGTTTCATCAA 480

5 CCCCAAACCA ATTACGTATC TGGAAGTTAT CAATACCGTG GCACAGGTCA CTTTGTGACAT 540
 TTTAATTTAT TACTTTTTGG GAATTAAATC CTTAGTCTAC ATGTTGGCAG CATCTTTACT 600
 TGGCCTGGGT TTGCACCCAA TTTCTGGACA TTTTATAGCT GAGCATTACA TGTCTTTAAA 660
 GGGTCATGAA ACTTACTCAT ATTATGGGCC TCTGAATTTA CTTACCTTCA ATGTGGGTTA 720
 10 TCATAATGAA CATCATGATT TCCCCAACAT TCCTGGAAAA AGTCTTCCAC TGGTGAGGAA 780
 AATAGCAGCT GAATACTATG ACAACCTCCC TCACTACAAT TCCTGGATAA AAGTACTGTA 840
 15 TGATTTTGTG ATGGATGATA CAATAAGTCC CTA CTCAAGA ATGAAGAGGC ACCAAAAAGG 900
 AGAGATGGTG CTGGAGTAAA TATCATTAGT GCCAAAGGGA TTCTTCTCCA AAACCTTTAGA 960
 TGATAAAATG GAATTTTTGC ATTATTAAAC TTGAGACCAG TGATGCTCAG AAGCTCCCCT 1020
 20 GGCACAATTT CAGAGTAAGA GCTCGGTGAT ACCAAGAAGT GAATCTGGCT TTTAAACAGT 1080
 CAGCCTGACT CTGTACTGCT CAGTTTCACT CACAGGAAAC TTGTGACTTG TGTATTATCG 1140
 25 TCATTGAGGA TGTTTCACTC ATGTCTGTCA TTTTATAAGC ATATCATTTA AAAAGCTTCT 1200
 AAAAAGCTAT TTCGCCAGG 1219

30 (2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 655 base pairs
 (B) TYPE: nucleic acid
 35 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2153526)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

45 TTACCTTCTA CGTCCGCTTC TTCCTCACTT ATGTGCCACT ATTGGGGCTG AAAGCTTCCT 60
 GGGCCTTTTC TTCATAGTCA GGTTCTGGA AAGCAACTGG TTTGTGTGGG TGACACAGAT 120
 GAACCATATT CCCATGCACA TTGATCATGA CCGGAACATG GACTGGGTTT CCACCCAGCT 180
 50 CCAGGCCACA TGCAATGTCC ACAAGTCTGC CTTCAATGAC TGGTTCAGTG GACACCTCAA 240
 CTTCCAGATT GAGCACCATC TTTTCCAC GATGCCTCGA CACAATTACC ACAAAGTGGC 300
 55 TCCCCTGGTG CAGTCCTTGT GTGCCAAGCA TGGCATAGAG TACCAGTCCA AGCCCTGCT 360
 GTCAGCCTTC GCCGACATCA TCCACTCACT AAAGGAGTCA GGGCAGCTCT GGCTAGATGC 420
 CTATCTTCAC CAATAACAAC AGCCACCCTG CCCAGTCTGG AAGAAGAGGA GGAAGACTCT 480
 60 GGAGCCAAGG CAGAGGGGAG CTTGAGGGAC AATGCCACTA TAGTTTAATA CTCAGAGGGG 540
 GTTGGGTTTG GGGACATAAA GCCTCTGACT CAAACTCCTC CCTTTTATCT TCTAGCCACA 600

GTTCTAAGAC CCAAAGTGGG GGGTGGACAC AGAAGTCCCT AGGAGGGAAG GAGCT 655

5 (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 304 base pairs
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3506132)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCTTTTACT TTGGCAATGG CTGGATTCCCT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60
TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120
CCCAAGTGGA ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCTCTGCC 180
AACTGGTGGA ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240
25 CCCGATGTGA ACATGCTGCA CGTGTTTGT CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300
AAGA 304

30 (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 918 base pairs
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3854933)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAGGGACCTA CCCC GCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGCGAG 60
GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120
45 CCAGGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180
GCCTTCCACA TCAACAAGGG CTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240
50 CTGTCTCCAG AGCAGCCCAG CTTTGAGCCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300
CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360
CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CTTTGGGTC 420
55 TTTGGGACGT CCTTTTGGC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGGCC 480
CAGGCTGGCT GGCTGCAGCA TGACTTTGGG CACCTGTCGG TCTTCAGCAC CTCAAAGTGG 540
60 AACCATCTGC TACATCATTT TGTGATTGGC CACCTGAAGG GGGCCCCCGC CAGTTGGTGG 600
AACCACATGC ACTTCCAGCA CCATGCCAAG CCCAACTGCT TCCGCAAAGA CCCAGACATC 660

AACATGCATC CCTTCTTCTT TGCCTTGGGG AAGATCCTCT CTGTGGAGCT TGGGAAACAG 720
 5 AAGAAAAAAT ATATGCCGTA CAACCACCAG CACARATACT TCTTCCTAAT TGGGCCCCCA 780
 GCCTTGCTGC CTCTCTACTT CCAGTGGTAT ATTTTCTATT TTGTTATCCA GCGAAAGAAG 840
 TGGGTGGACT TGGCCTGGAT CAGCAAACAG GAATACGATG AAGCCGGGCT TCCATTGTCC 900
 10 ACCGCAAATG CTTCTAAA 918

(2) INFORMATION FOR SEQ ID NO:35:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1686 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 20 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2511785)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
 25 GCCACTTAAA GGGTGCCTCT GCCAACTGGT GGAATCATCG CCACTTCCAG CACCACGCCA 60
 AGCCTAACAT CTTCCACAAG GATCCCGATG TGAACATGCT GCACGTGTTT GTTCTGGGCG 120
 30 AATGGCAGCC CATCGAGTAC GGCAAGAAGA AGCTGAAATA CCTGCCCTAC AATCACCAGC 180
 ACGAATACTT CTTCTGATT GGGCCGCCGC TGCTCATCCC CATGTATTTC CAGTACCAGA 240
 35 TCATCATGAC CATGATCGTC CATAAGAACT GGGTGGACCT GGCCTGGGCC GTCAGCTACT 300
 ACATCCGGTT CTTTCATACC TACATCCCTT TCTACGGCAT CCTGGGAGCC CTCCTTTTCC 360
 TCAACTTCAT CAGGTTCTTG GAGAGCCACT GGTTTGTGTG GGTCACACAG ATGAATCACA 420
 40 TCGTCATGGA GATTGACCAG GAGGCCTACC GTGACTGGTT CAGTAGCCAG CTGACAGCCA 480
 CCTGCAACGT GGAGCAGTCC TTCTTCAACG ACTGGTTCAG TGGACACCTT AACTTCCAGA 540
 45 TTGAGCACCA CCTCTTCCCC ACCATGCCCC GGCACAACCT ACACAAGATC GCCCCGCTGG 600
 TGAAGTCTCT ATGTGCCAAG CATGGCATTG AATACCAGGA GAAGCCGCTA CTGAGGGCCC 660
 TGCTGGACAT CATCAGGTCC CTGAAGAAGT CTGGGAAGCT GTGGCTGGAC GCCTACCTTC 720
 50 ACAAATGAAG CCACAGCCCC CGGGACACCG TGGGGAAGGG GTGCAGGTGG GGTGATGGCC 780
 AGAGGAATGA TGGGCTTTTG TTCTGAGGGG TGTCCGAGAG GCTGGTGTAT GCACTGCTCA 840
 55 CGGACCCCAT GTTGGATCTT TCTCCCTTTC TCCTCTCCTT TTTCTCTTCA CATCTCCCCC 900
 ATAGCACCTT GCCCTCATGG GACCTGCCCT CCCTCAGCCG TCAGCCATCA GCCATGGCCC 960
 TCCCAGTGCC TCCTAGCCCC TTCTTCCAAG GAGCAGAGAG GTGGCCACCG GGGGTGGCTC 1020
 60 TGTCCTACCT CCACTCTCTG CCCCTAAAGA TGGGAGGAGA CCAGCGGTCC ATGGGTCTGG 1080
 CCTGTGAGTC TCCCCTTGCA GCCTGGTCAC TAGGCATCAC CCCCCTTTG GTTCTTCAGA 1140

5 TGCTCTTGGG GTTCATAGGG GCAGGTCTTA GTCGGGCAGG GCCCCTGACC CTCCCGGCCT 1200
 GGCTTCACTC TCCCTGACGG CTGCCATTGG TCCACCCTTT CATAGAGAGG CCTGCTTTGT 1260
 TACAAAGCTC GGGTCTCCCT CCTGCAGCTC GGTTAAGTAC CCGAGGCCTC TCTTAAGATG 1320
 TCCAGGGCCC CAGGCCCCGG GGCACAGCCA GCCCAAACCT TGGGCCCTGG AAGAGTCCTC 1380
 10 CACCCCATCA CTAGAGTGCT CTGACCCTGG GCTTTCACGG GCCCCATTCC ACCGCCTCCC 1440
 CAACTTGAGC CTGTGACCTT GGGACCAAAG GGGGAGTCCC TCGTCTCTTG TGA CTCAGCA 1500
 15 GAGGCAGTGG CCACGTTT CAG GGAGGGGCG GCTGGCCTGG AGGCTCAGCC CACCCTCCAG 1560
 CTTTTCTCA GGGTGTCTG AGGTCCAAGA TTCTGGAGCA ATCTGACCCT TCTCAAAGG 1620
 CTCTGTTATC AGCTGGGCAG TGCCAGCCAA TCCCTGGCCA TTTGGCCCCA GGGGACGTGG 1680
 20 GCCCTG 1686

(2) INFORMATION FOR SEQ ID NO:36:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1843 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 30 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid (Contig 2535)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

35 GTCTTTTACT TTGGCAATGG CTGGATTCTT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60
 TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120
 40 CCCAAGTGGA ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCCTTGCC 180
 AACTGGTGGA ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240
 45 CCCGATGTGA ACATGCTGCA CGTGTTTGTT CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300
 AAGAAGAAGC TGAATACCT GCCCTACAAT CACCAGCACG AATACTTCTT CCTGATTGGG 360
 CCGCCGCTGC TCATCCCCAT GTATTTCCAG TACCAGATCA TCATGACCAT GATCGTCCAT 420
 50 AAGAACTGGG TGGACCTGGC CTGGGCCGTC AGCTACTACA TCCGGTTCTT CATCACCTAC 480
 ATCCCTTTCT ACGGCATCCT GGGAGCCCTC CTTTTCTCA ACTTCATCAG GTTCCTGGAG 540
 55 AGCCACTGGT TTGTGTGGGT CACACAGATG AATCACATCG TCATGGAGAT TGACCAGGAG 600
 GCCTACCGTG ACTGGTTCAG TAGCCAGCTG ACAGCCACCT GCAACGTGGA GCAGTCCTTC 660
 TTCAACGACT GGTTCAGTGG ACACCTTAAC TTCCAGATTG AGCACCACCT CTTCCCCACC 720
 60 ATGCCCCGGC ACAACTTACA CAAGATCGCC CCGCTGGTGA AGTCTCTATG TGCCAAGCAT 780
 GGCATTGAAT ACCAGGAGAA GCCGCTACTG AGGGCCCTGC TGGACATCAT CAGGTCCCTG 840

5 AAGAAGTCTG GGAAGCTGTG GCTGGACGCC TACCTTCACA AATGAAGCCA CAGCCCCCGG 900
 GACACCGTGG GGAAGGGGTG CAGGTGGGGT GATGGCCAGA GGAATGATGG GCTTTTGTTC 960
 TGAGGGGTGT CCGAGAGGCT GGTGTATGCA CTGCTCACGG ACCCCATGTT GGATCTTTCT 1020
 10 CCCTTTCTCC TCTCCTTTTT CTCTTCACAT CTCCCCATA GCACCCTGCC CTCATGGGAC 1080
 CTGCCCTCCC TCAGCCGTCA GCCATCAGCC ATGGCCCTCC CAGTGCCTCC TAGCCCTTC 1140
 TTCCAAGGAG CAGAGAGGTG GCCACCGGGG GTGGCTCTGT CCTACCTCCA CTCTCTGCCC 1200
 15 CTAAAGATGG GAGGAGACCA GCGGTCCATG GGTCTGGCCT GTGAGTCTCC CCTTGCAGCC 1260
 TGGTCACTAG GCATCACCCC CGCTTTGGTT CTTCAGATGC TCTTGGGGTT CATAGGGGCA 1320
 GGTCTAGTGC GGGCAGGGCC CCTGACCCTC CCGGCCTGGC TTCACTCTCC CTGACGGCTG 1380
 20 CCATTGGTCC ACCCTTTCAT AGAGAGGCCT GCTTTGTTAC AAAGCTCGGG TCTCCCTCCT 1440
 GCAGCTCGGT TAAGTACCCG AGGCCTCTCT TAAGATGTCC AGGGCCCCAG GCCGCGGGC 1500
 25 ACAGCCAGCC CAAACCTTGG GCCCTGGAAG AGTCTCCAC CCCATCACTA GAGTGCTCTG 1560
 ACCCTGGGCT TTCACGGGCC CCATTCCACC GCCTCCCCAA CTTGAGCCTG TGACCTTGGG 1620
 ACCAAAGGGG GAGTCCCTCG TCTCTTGTGA CTCAGCAGAG GCAGTGGCCA CGTTCAGGGA 1680
 30 GGGGCCGGCT GGCCTGGAGG CTCAGCCAC CCTCCAGCTT TTCCTCAGGG TGTCTGAGG 1740
 TCCAAGATTC TGGAGCAATC TGACCCTTCT CCAAAGGCTC TGTTATCAGC TGGGCAGTGC 1800
 35 CAGCCAATCC CTGGCCATTT GGCCCCAGGG GACGTGGGCC CTG 1843

(2) INFORMATION FOR SEQ ID NO:37:

40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2257 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 45 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 253538a)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
 50 CAGGGACCTA CCCC GCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGGCAG 60
 GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120
 CCAGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180
 55 GCCTTCCACA TCAACAAGGG CCTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240
 CTGTCTCCAG AGCAGCCCAG CTTTGGAGCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300
 CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360
 60 CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CTTTGGGTC 420

| | | |
|----|--|------|
| | TTTGGGACGT CCTTTTGGCC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGCAG | 480 |
| | GCCCAAGCTG GATGGCTGCA ACATGATTAT GGCCACCTGT CTGTCTACAG AAAACCCAAG | 540 |
| 5 | TGGAACCACC TTGTCCACAA ATTCGTCATT GGCCACTTAA AGGGTGCCTC TGCCAACTGG | 600 |
| | TGGAATCATC GCCACTTCCA GCACCAGGCC AAGCCTAACA TCTTCCACAA GGATCCCGAT | 660 |
| 10 | GTGAACATGC TGCACGTGTT TGTTCTGGGC GAATGGCAGC CCATCGAGTA CGGCAAGAAG | 720 |
| | AAGCTGAAAT ACCTGCCCTA CAATCACCAG CACGAATACT TCTTCCTGAT TGGGCCGCCG | 780 |
| | CTGCTCATCC CCATGTATTT CCAGTACCAG ATCATCATGA CCATGATCGT CCATAAGAAC | 840 |
| 15 | TGGGTGGACC TGGCCTGGGC CGTCAGCTAC TACATCCGGT TCTTCATCAC CTACATCCCT | 900 |
| | TTCTACGGCA TCCTGGGAGC CCTCCTTTTC CTCAACTTCA TCAGGTTTCCT GGAGAGCCAC | 960 |
| 20 | TGGTTTGTGT GGGTCACACA GATGAATCAC ATCGTCATGG AGATTGACCA GGAGGCCTAC | 1020 |
| | CGTGA CTGGT TCAGTAGCCA GCTGACAGCC ACCTGCAACG TGGAGCAGTC CTTCTTCAAC | 1080 |
| | GACTGGTTCA GTGGACACCT TAACTTCCAG ATTGAGCACC ACCTCTTCCC CACCATGCCC | 1140 |
| 25 | CGGCACAAC TACACAAGAT CGCCCCGCTG GTGAAGTCTC TATGTGCCAA GCATGGCATT | 1200 |
| | GAATACCAGG AGAAGCCGCT ACTGAGGGCC CTGCTGGACA TCATCAGGTC CCTGAAGAAG | 1260 |
| 30 | TCTGGGAAGC TGTGGCTGGA CGCCTACCTT CACAAATGAA GCCACAGCCC CCGGGACACC | 1320 |
| | GTGGGGAAGG GGTGCAGGTG GGGTGATGGC CAGAGGAATG ATGGGCTTTT GTTCTGAGGG | 1380 |
| | GTGTCCGAGA GGCTGGTGTA TGCAC TGCTC ACGGACCCCA TGTTGGATCT TTCTCCCTTT | 1440 |
| 35 | CTCCTCTCCT TTTTCTCTTC ACATCTCCCC CATAGCACCC TGCCCTCATG GGACCTGCCC | 1500 |
| | TCCCTCAGCC GTCAGCCATC AGCCATGGCC CTCCCAGTGC CTCCTAGCCC CTTCTTCCAA | 1560 |
| 40 | GGAGCAGAGA GGTGGCCACC GGGGGTGGCT CTGTCTTACC TCCACTCTCT GCCCCTAAAG | 1620 |
| | ATGGGAGGAG ACCAGCGGTC CATGGGTCTG GCCTGTGAGT CTCCCCTTGC AGCCTGGTCA | 1680 |
| | CTAGGCATCA CCCCCGCTTT GGTTCCTCAG ATGCTCTTGG GGTTCATAGG GGCAGGTCCT | 1740 |
| 45 | AGTCGGGCAG GGCCCTGAC CCTCCCGGCC TGGCTTCACT CTCCCTGACG GCTGCCATTG | 1800 |
| | GTCCACCCTT TCATAGAGAG GCCTGCTTTG TTACAAAGCT CGGGTCTCCC TCCTGCAGCT | 1860 |
| 50 | CGGTAAAGTA CCCGAGGCCT CTCTTAAGAT GTCCAGGGCC CCAGGCCCGC GGGCACAGCC | 1920 |
| | AGCCCAAACC TTGGGCCCTG GAAGAGTCCT CCACCCCATC ACTAGAGTGC TCTGACCCTG | 1980 |
| | GGCTTTTACG GGCCCCATTC CACCGCCTCC CCAACTTGAG CCTGTGACCT TGGGACCAAA | 2040 |
| 55 | GGGGGAGTCC CTCGTCTCTT GTGACTCAGC AGAGGCAGTG GCCACGTTCA GGGAGGGGCC | 2100 |
| | GGCTGGCCTG GAGGCTCAGC CCACCCTCCA GCTTTTCCTC AGGGTGTCTT GAGGTCCAAG | 2160 |
| 60 | ATTCTGGAGC AATCTGACCC TTCTCCAAAG GCTCTGTTAT CAGCTGGGCA GTGCCAGCCA | 2220 |
| | ATCCCTGGCC ATTTGGCCCC AGGGGACGTG GGCCCTG | 2257 |

(2) INFORMATION FOR SEQ ID NO:38:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 411 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: amino acid (Translation of Contig 2692004)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

| | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| 15 | His | Ala | Asp | Arg | Arg | Arg | Glu | Ile | Leu | Ala | Lys | Tyr | Pro | Glu | Ile | 1 | 5 | 10 | 15 |
| | Lys | Ser | Leu | Met | Lys | Pro | Asp | Pro | Asn | Leu | Ile | Trp | Ile | Ile | Ile | 20 | 25 | 30 | |
| 20 | Met | Met | Val | Leu | Thr | Gln | Leu | Gly | Ala | Phe | Tyr | Ile | Val | Lys | Asp | 35 | 40 | 45 | |
| | Leu | Asp | Trp | Lys | Trp | Val | Ile | Phe | Gly | Ala | Tyr | Ala | Phe | Gly | Ser | 50 | 55 | 60 | |
| | Cys | Ile | Asn | His | Ser | Met | Thr | Leu | Ala | Ile | His | Glu | Ile | Ala | His | 65 | 70 | 75 | |
| 25 | Asn | Ala | Ala | Phe | Gly | Asn | Cys | Lys | Ala | Met | Trp | Asn | Arg | Trp | Phe | 80 | 85 | 90 | |
| | Gly | Met | Phe | Ala | Asn | Leu | Pro | Ile | Gly | Ile | Pro | Tyr | Ser | Ile | Ser | 95 | 100 | 105 | |
| 30 | Phe | Lys | Arg | Tyr | His | Met | Asp | His | His | Arg | Tyr | Leu | Gly | Ala | Asp | 110 | 115 | 120 | |
| | Gly | Val | Asp | Val | Asp | Ile | Pro | Thr | Asp | Phe | Glu | Gly | Trp | Phe | Phe | 125 | 130 | 135 | |
| | Cys | Thr | Ala | Phe | Arg | Lys | Phe | Ile | Trp | Val | Ile | Leu | Gln | Pro | Leu | 140 | 145 | 150 | |
| 35 | Phe | Tyr | Ala | Phe | Arg | Pro | Leu | Phe | Ile | Asn | Pro | Lys | Pro | Ile | Thr | 155 | 160 | 165 | |
| | Tyr | Leu | Glu | Val | Ile | Asn | Thr | Val | Ala | Gln | Val | Thr | Phe | Asp | Ile | 170 | 175 | 180 | |
| 40 | Leu | Ile | Tyr | Tyr | Phe | Leu | Gly | Ile | Lys | Ser | Leu | Val | Tyr | Met | Leu | 185 | 190 | 195 | |
| | Ala | Ala | Ser | Leu | Leu | Gly | Leu | Gly | Leu | His | Pro | Ile | Ser | Gly | His | 200 | 205 | 210 | |
| | Phe | Ile | Ala | Glu | His | Tyr | Met | Phe | Leu | Lys | Gly | His | Glu | Thr | Tyr | 215 | 220 | 225 | |
| 45 | Ser | Tyr | Tyr | Gly | Pro | Leu | Asn | Leu | Leu | Thr | Phe | Asn | Val | Gly | Tyr | 230 | 235 | 240 | |
| | His | Asn | Glu | His | His | Asp | Phe | Pro | Asn | Ile | Pro | Gly | Lys | Ser | Leu | 245 | 250 | 255 | |
| 50 | Pro | Leu | Val | Arg | Lys | Ile | Ala | Ala | Glu | Tyr | Tyr | Asp | Asn | Leu | Pro | 260 | 265 | 270 | |
| | His | Tyr | Asn | Ser | Trp | Ile | Lys | Val | Leu | Tyr | Asp | Phe | Val | Met | Asp | 275 | 280 | 285 | |
| | Asp | Thr | Ile | Ser | Pro | Tyr | Ser | Arg | Met | Lys | Arg | His | Gln | Lys | Gly | 290 | 295 | 300 | |
| 55 | Glu | Met | Val | Leu | Glu | *** | Ile | Ser | Leu | Val | Pro | Lys | Gly | Phe | Phe | 305 | 310 | 315 | |
| | Ser | Lys | Thr | Leu | Asp | Lys | Met | Glu | Phe | Leu | His | Tyr | *** | Thr | | 320 | 325 | 330 | |
| | *** | Asp | Gln | *** | Cys | Ser | Glu | Ala | Pro | Leu | Ala | Gln | Phe | Gln | Ser | 335 | 340 | 345 | |
| 60 | Lys | Ser | Ser | Val | Ile | Pro | Arg | Ser | Glu | Ser | Gly | Phe | *** | Thr | Val | 350 | 355 | 360 | |

Ser Leu Thr Leu Tyr Cys Ser Val Ser Leu Thr Gly Asn Leu ***
 365 370 375
 Leu Val Tyr Tyr Arg His *** Gly Cys Phe Thr His Val Cys His
 380 385 390
 5 Phe Ile Ser Ile Ser Phe Lys Lys Leu Leu Lys Ser Tyr Phe Ala
 400 405 410
 Arg

10 (2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 218 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2153526)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Tyr Leu Leu Arg Pro Leu Leu Pro His Leu Cys Ala Thr Ile Gly
 1 5 10 15
 25 Ala Glu Ser Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu
 20 25 30
 Ser Asn Trp Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met
 35 40 45
 His Ile Asp His Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu
 50 55 60
 30 Gln Ala Thr Cys Asn Val His Lys Ser Ala Phe Asn Asp Trp Phe
 65 70 75
 Ser Gly His Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
 80 85 90
 35 Met Pro Arg His Asn Tyr His Lys Val Ala Pro Leu Val Gln Ser
 95 100 105
 Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser Lys Pro Leu Leu
 110 115 120
 Ser Ala Phe Ala Asp Ile Ile His Ser Leu Lys Glu Ser Gly Gln
 125 130 135
 40 Leu Trp Leu Asp Ala Tyr Leu His Gln *** Gln Gln Pro Pro Cys
 140 145 150
 Pro Val Trp Lys Lys Arg Arg Lys Thr Leu Glu Pro Arg Gln Arg
 155 160 165
 45 Gly Ala *** Gly Thr Met Pro Leu *** Phe Asn Thr Gln Arg Gly
 170 175 180
 Leu Gly Leu Gly Thr *** Ser Leu *** Leu Lys Leu Leu Pro Phe
 185 190 195
 Ile Phe *** Pro Gln Phe *** Asp Pro Lys Trp Gly Val Asp Thr
 200 205 210
 50 Glu Val Pro Arg Arg Glu Gly Ala
 215

55 (2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 3506132)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

5

```

Val Phe Tyr Phe Gly Asn Gly Trp Ile Pro Thr Leu Ile Thr Ala
1      5      10      15
Phe Val Leu Ala Thr Ser Gln Ala Gln Ala Gly Trp Leu Gln His
10     20     25     30
Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys Trp Asn His
      35     40     45
Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly Ala Ser Ala
      50     55     60
15    Asn Trp Trp Asn His Arg His Phe Gln His His Ala Lys Pro Asn
      65     70     75
Leu Gly Glu Trp Gln Pro Ile Glu Tyr Gly Lys Xxx
      80     85

```

20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 306 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 3854933)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln
35 1      5      10      15
Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val
      20     25     30
40  Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg
      35     40     45
Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val
      50     55     60
Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser
      65     70     75
45  Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro
      80     85     90
Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala
      95    100    105
50  Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe
      110   115   120
Leu Leu Tyr Leu Leu His Ile Leu Leu Asp Gly Ala Ala Trp
      125   130   135
Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu
      140   145   150
55  Cys Ala Val Leu Leu Ser Ala Val Gln Ala Gln Ala Gly Trp Leu
      155   160   165
Gln His Asp Phe Gly His Leu Ser Val Phe Ser Thr Ser Lys Trp
      170   175   180
60  Asn His Leu Leu His His Phe Val Ile Gly His Leu Lys Gly Ala
      185   190   195
Pro Ala Ser Trp Trp Asn His Met His Phe Gln His His Ala Lys
      200   205   210

```

5 Pro Asn Cys Phe Arg Lys Asp Pro Asp Ile Asn Met His Pro Phe
 215 220 225
 Phe Phe Ala Leu Gly Lys Ile Leu Ser Val Glu Leu Gly Lys Gln
 230 235 240
 Lys Lys Lys Tyr Met Pro Tyr Asn His Gln His Xxx Tyr Phe Phe
 245 250 255
 Leu Ile Gly Pro Pro Ala Leu Leu Pro Leu Tyr Phe Gln Trp Tyr
 260 265 270
 10 Ile Phe Tyr Phe Val Ile Gln Arg Lys Lys Trp Val Asp Leu Ala
 275 280 285
 Trp Ile Ser Lys Gln Glu Tyr Asp Glu Ala Gly Leu Pro Leu Ser
 290 295 300
 Thr Ala Asn Ala Ser Lys
 305

15

(2) INFORMATION FOR SEQ ID NO:42:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 566 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: amino acid (Translation of Contig 2511785)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

30 His Leu Lys Gly Ala Ser Ala Asn Trp Trp Asn His Arg His Phe
 1 5 10 15
 Gln His His Ala Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val
 20 25 30
 35 Asn Met Leu His Val Phe Val Leu Gly Glu Trp Gln Pro Ile Glu
 35 40 45
 Tyr Gly Lys Lys Lys Leu Lys Tyr Leu Pro Tyr Asn His Gln His
 50 55 60
 Glu Tyr Phe Phe Leu Ile Gly Pro Pro Leu Leu Ile Pro Met Tyr
 65 70 75
 40 Phe Gln Tyr Gln Ile Ile Met Thr Met Ile Val His Lys Asn Trp
 80 85 90
 Val Asp Leu Ala Trp Ala Val Ser Tyr Tyr Ile Arg Phe Phe Ile
 95 100 105
 45 Thr Tyr Ile Pro Phe Tyr Gly Ile Leu Gly Ala Leu Leu Phe Leu
 110 115 120
 Asn Phe Ile Arg Phe Leu Glu Ser His Trp Phe Val Trp Val Thr
 125 130 135
 Gln Met Asn His Ile Val Met Glu Ile Asp Gln Glu Ala Tyr Arg
 140 145 150
 50 Asp Trp Phe Ser Ser Gln Leu Thr Ala Thr Cys Asn Val Glu Gln
 155 160 165
 Ser Phe Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile
 170 175 180
 55 Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu His Lys
 185 190 195
 Ile Ala Pro Leu Val Lys Ser Leu Cys Ala Lys His Gly Ile Glu
 200 205 210
 Tyr Gln Glu Lys Pro Leu Leu Arg Ala Leu Leu Asp Ile Ile Arg
 215 220 225
 60 Ser Leu Lys Lys Ser Gly Lys Leu Trp Leu Asp Ala Tyr Leu His
 230 235 240
 Lys *** Ser His Ser Pro Arg Asp Thr Val Gly Lys Gly Cys Arg

| | | | | | | |
|----|-----------------|---------------------|---------------------|-----|--|-----|
| | | 245 | | 250 | | 255 |
| | Trp Gly Asp Gly | Gln Arg Asn Asp Gly | Leu Leu Ph *** Gly | Val | | |
| | | 260 | | 265 | | 270 |
| 5 | Ser Glu Arg Leu | Val Tyr Ala Leu Leu | Thr Asp Pro Met Leu | Asp | | |
| | | 275 | | 280 | | 285 |
| | Leu Ser Pro Phe | Leu Leu Ser Phe Phe | Ser Ser His Leu Pro | His | | |
| | | 290 | | 295 | | 300 |
| | Ser Thr Leu Pro | Ser Trp Asp Leu Pro | Ser Leu Ser Arg Gln | Pro | | |
| | | 305 | | 310 | | 315 |
| 10 | Ser Ala Met Ala | Leu Pro Val Pro Pro | Ser Pro Phe Phe Gln | Gly | | |
| | | 320 | | 325 | | 330 |
| | Ala Glu Arg Trp | Pro Pro Gly Val Ala | Leu Ser Tyr Leu His | Ser | | |
| | | 335 | | 340 | | 345 |
| 15 | Leu Pro Leu Lys | Met Gly Gly Asp Gln | Arg Ser Met Gly Leu | Ala | | |
| | | 350 | | 355 | | 360 |
| | Cys Glu Ser Pro | Leu Ala Ala Trp Ser | Leu Gly Ile Thr Pro | Ala | | |
| | | 365 | | 370 | | 375 |
| | Leu Val Leu Gln | Met Leu Leu Gly Phe | Ile Gly Ala Gly Pro | Ser | | |
| | | 380 | | 385 | | 390 |
| 20 | Arg Ala Gly Pro | Leu Thr Leu Pro Ala | Trp Leu His Ser Pro | *** | | |
| | | 400 | | 405 | | 410 |
| | Arg Leu Pro Leu | Val His Pro Phe Ile | Glu Arg Pro Ala Leu | Leu | | |
| | | 415 | | 420 | | 425 |
| 25 | Gln Ser Ser Gly | Leu Pro Pro Ala Ala | Arg Leu Ser Thr Arg | Gly | | |
| | | 430 | | 435 | | 440 |
| | Leu Ser *** Asp | Val Gln Gly Pro Arg | Pro Ala Gly Thr Ala | Ser | | |
| | | 445 | | 450 | | 455 |
| | Pro Asn Leu Gly | Pro Trp Lys Ser Pro | Pro Pro His His *** | Ser | | |
| | | 460 | | 465 | | 470 |
| 30 | Ala Leu Thr Leu | Gly Phe His Gly Pro | His Ser Thr Ala Ser | Pro | | |
| | | 475 | | 480 | | 485 |
| | Thr *** Ala Cys | Asp Leu Gly Thr Lys | Gly Gly Val Pro Arg | Leu | | |
| | | 490 | | 495 | | 500 |
| 35 | Leu *** Leu Ser | Arg Gly Ser Gly His | Val Gln Gly Gly Ala | Gly | | |
| | | 505 | | 510 | | 515 |
| | Trp Pro Gly Gly | Ser Ala His Pro Pro | Ala Phe Pro Gln Gly | Val | | |
| | | 520 | | 525 | | 530 |
| | Leu Arg Ser Lys | Ile Leu Glu Gln Ser | Asp Pro Ser Pro Lys | Ala | | |
| | | 535 | | 540 | | 545 |
| 40 | Leu Leu Ser Ala | Gly Gln Cys Gln Pro | Ile Pro Gly His Leu | Ala | | |
| | | 550 | | 555 | | 560 |
| | Pro Gly Asp Val | Gly Pro Xxx | | | | |
| | | 565 | | | | |

45

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 619 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2535)

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

60

| | | | |
|---|---|----|----|
| Val Phe Tyr Phe Gly Asn Gly Trp Ile Pro Thr Leu Ile Thr Ala | | | |
| 1 | 5 | 10 | 15 |
| Phe Val Leu Ala Thr Ser Gln Ala Gln Ala Gly Trp Leu Gln His | | | |

| | | | | | | |
|----|-----------------|-------------------------|---------------------|-----|--|-----|
| | | 20 | | 25 | | 30 |
| | Asp Tyr Gly His | Leu Ser Val Tyr Arg Lys | Pro Lys Trp Asn His | | | |
| | | 35 | | 40 | | 45 |
| 5 | Leu Val His Lys | Phe Val Ile Gly His Leu | Lys Gly Ala Ser Ala | | | |
| | | 50 | | 55 | | 60 |
| | Asn Trp Trp Asn | His Arg His Phe Gln His | His Ala Lys Pro Asn | | | |
| | | 65 | | 70 | | 75 |
| | Ile Phe His Lys | Asp Pro Asp Val Asn Met | Leu His Val Phe Val | | | |
| | | 80 | | 85 | | 90 |
| 10 | Leu Gly Glu Trp | Gln Pro Ile Glu Tyr Gly | Lys Lys Lys Leu Lys | | | |
| | | 95 | | 100 | | 105 |
| | Tyr Leu Pro Tyr | Asn His Gln His Glu Tyr | Phe Phe Leu Ile Gly | | | |
| | | 110 | | 115 | | 120 |
| 15 | Pro Pro Leu Leu | Ile Pro Met Tyr Phe Gln | Tyr Gln Ile Ile Met | | | |
| | | 125 | | 130 | | 135 |
| | Thr Met Ile Val | His Lys Asn Trp Val Asp | Leu Ala Trp Ala Val | | | |
| | | 140 | | 145 | | 150 |
| | Ser Tyr Tyr Ile | Arg Phe Phe Ile Thr Tyr | Ile Pro Phe Tyr Gly | | | |
| | | 155 | | 160 | | 165 |
| 20 | Ile Leu Gly Ala | Leu Leu Phe Leu Asn Phe | Ile Arg Phe Leu Glu | | | |
| | | 170 | | 175 | | 180 |
| | Ser His Trp Phe | Val Trp Val Thr Gln Met | Asn His Ile Val Met | | | |
| | | 185 | | 190 | | 195 |
| 25 | Glu Ile Asp Gln | Glu Ala Tyr Arg Asp Trp | Phe Ser Ser Gln Leu | | | |
| | | 200 | | 205 | | 210 |
| | Thr Ala Thr Cys | Asn Val Glu Gln Ser Phe | Phe Asn Asp Trp Phe | | | |
| | | 215 | | 220 | | 225 |
| | Ser Gly His Leu | Asn Phe Gln Ile Glu His | His Leu Phe Pro Thr | | | |
| | | 230 | | 235 | | 240 |
| 30 | Met Pro Arg His | Asn Leu His Lys Ile Ala | Pro Leu Val Lys Ser | | | |
| | | 245 | | 250 | | 255 |
| | Leu Cys Ala Lys | His Gly Ile Glu Tyr Gln | Glu Lys Pro Leu Leu | | | |
| | | 260 | | 265 | | 270 |
| 35 | Arg Ala Leu Leu | Asp Ile Ile Arg Ser Leu | Lys Lys Ser Gly Lys | | | |
| | | 275 | | 280 | | 285 |
| | Leu Trp Leu Asp | Ala Tyr Leu His Lys *** | Ser His Ser Pro Arg | | | |
| | | 290 | | 295 | | 300 |
| | Asp Thr Val Gly | Lys Gly Cys Arg Trp Gly | Asp Gly Gln Arg Asn | | | |
| | | 305 | | 310 | | 315 |
| 40 | Asp Gly Leu Leu | Phe *** Gly Val Ser Glu | Arg Leu Val Tyr Ala | | | |
| | | 320 | | 325 | | 330 |
| | Leu Leu Thr Asp | Pro Met Leu Asp Leu Ser | Pro Phe Leu Leu Ser | | | |
| | | 335 | | 340 | | 345 |
| 45 | Phe Phe Ser Ser | His Leu Pro His Ser Thr | Leu Pro Ser Trp Asp | | | |
| | | 350 | | 355 | | 360 |
| | Leu Pro Ser Leu | Ser Arg Gln Pro Ser Ala | Met Ala Leu Pro Val | | | |
| | | 365 | | 370 | | 375 |
| | Pro Pro Ser Pro | Phe Phe Gln Gly Ala Glu | Arg Trp Pro Pro Gly | | | |
| | | 380 | | 385 | | 390 |
| 50 | Val Ala Leu Ser | Tyr Leu His Ser Leu Pro | Leu Lys Met Gly Gly | | | |
| | | 400 | | 405 | | 410 |
| | Asp Gln Arg Ser | Met Gly Leu Ala Cys Glu | Ser Pro Leu Ala Ala | | | |
| | | 415 | | 420 | | 425 |
| 55 | Trp Ser Leu Gly | Ile Thr Pro Ala Leu Val | Leu Gln Met Leu Leu | | | |
| | | 430 | | 435 | | 440 |
| | Gly Phe Ile Gly | Ala Gly Pro Ser Arg Ala | Gly Pro Leu Thr Leu | | | |
| | | 445 | | 450 | | 455 |
| | Pro Ala Trp Leu | His Ser Pro *** Arg Leu | Pro Leu Val His Pro | | | |
| | | 460 | | 465 | | 470 |
| 60 | Phe Ile Glu Arg | Pro Ala Leu Leu Gln Ser | Ser Gly Leu Pro Pro | | | |
| | | 475 | | 480 | | 485 |
| | Ala Ala Arg Leu | Ser Thr Arg Gly Leu Ser | *** Asp Val Gln Gly | | | |

```

      490                      495                      500
Pro Arg Pro Ala Gly Thr Ala Ser Pro Asn Leu Gly Pro Trp Lys
      505                      510                      515
5  Ser Pro Pro Pro His His *** Ser Ala Leu Thr Leu Gly Phe His
      520                      525                      530
Gly Pro His Ser Thr Ala Ser Pro Thr *** Ala Cys Asp Leu Gly
      535                      540                      545
Thr Lys Gly Gly Val Pro Arg Leu Leu *** Leu Ser Arg Gly Ser
      550                      555                      560
10 Gly His Val Gln Gly Ala Gly Trp Pro Gly Gly Ser Ala His
      565                      570                      575
Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys Ile Leu Glu
      580                      585                      590
15 Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala Gly Gln Cys
      595                      600                      605
Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val Gly Pro Xxx
      610                      615                      620

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20

(2) INFORMATION FOR SEQ ID NO:44:

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      (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 757 amino acids
25      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

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      (ii) MOLECULE TYPE: amino acid (Translation of Contig 253538a)
30

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```

35 Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln
   1      5      10      15
Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val
   20      25      30
Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg
   35      40      45
40 Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val
   50      55      60
Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser
   65      70      75
45 Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro
   80      85      90
Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala
   95      100      105
Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe
  110      115      120
50 Leu Leu Tyr Leu Leu His Ile Leu Leu Leu Asp Gly Ala Ala Trp
  125      130      135
Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu
  140      145      150
55 Cys Ala Val Leu Leu Ser Ala Val Gln Gln Ala Gln Ala Gly Trp
  155      160      165
Leu Gln His Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys
  170      175      180
Trp Asn His Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly
  185      190      195
60 Ala Ser Ala Asn Trp Trp Asn His Arg His Phe Gln His His Ala
  200      205      210
Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val Asn Met Leu His

```

| | | | | | | |
|----|---------------------|-----------------|---------------------|-----|--|-----|
| | | 215 | | 220 | | 225 |
| | Val Phe Val Leu Gly | Glu Trp Gln Pro | Ile Glu Tyr Gly Lys | Lys | | |
| | | 230 | | 235 | | 240 |
| 5 | Lys Leu Lys Tyr Leu | Pro Tyr Asn His | Gln His Glu Tyr Phe | Phe | | |
| | | 245 | | 250 | | 255 |
| | Leu Ile Gly Pro Pro | Leu Leu Ile Pro | Met Tyr Phe Gln Tyr | Gln | | |
| | | 260 | | 265 | | 270 |
| | Ile Ile Met Thr Met | Ile Val His Lys | Asn Trp Val Asp Leu | Ala | | |
| | | 275 | | 280 | | 285 |
| 10 | Trp Ala Val Ser Tyr | Tyr Ile Arg Phe | Phe Ile Thr Tyr Ile | Pro | | |
| | | 290 | | 295 | | 300 |
| | Phe Tyr Gly Ile Leu | Gly Ala Leu Leu | Phe Leu Asn Phe Ile | Arg | | |
| | | 305 | | 310 | | 315 |
| 15 | Phe Leu Glu Ser His | Trp Phe Val Trp | Val Thr Gln Met Asn | His | | |
| | | 320 | | 325 | | 330 |
| | Ile Val Met Glu Ile | Asp Gln Glu Ala | Tyr Arg Asp Trp Phe | Ser | | |
| | | 335 | | 340 | | 345 |
| | Ser Gln Leu Thr Ala | Thr Cys Asn Val | Glu Gln Ser Phe Phe | Asn | | |
| | | 350 | | 355 | | 360 |
| 20 | Asp Trp Phe Ser Gly | His Leu Asn Phe | Gln Ile Glu His His | Leu | | |
| | | 365 | | 370 | | 375 |
| | Phe Pro Thr Met Pro | Arg His Asn Leu | His Lys Ile Ala Pro | Leu | | |
| | | 380 | | 385 | | 390 |
| 25 | Val Lys Ser Leu Cys | Ala Lys His Gly | Ile Glu Tyr Gln Glu | Lys | | |
| | | 400 | | 405 | | 410 |
| | Pro Leu Leu Arg Ala | Leu Leu Asp Ile | Ile Arg Ser Leu Lys | Lys | | |
| | | 415 | | 420 | | 425 |
| | Ser Gly Lys Leu Trp | Leu Asp Ala Tyr | Leu His Lys *** | Ser | | |
| | | 430 | | 435 | | 440 |
| 30 | Ser Pro Arg Asp Thr | Val Gly Lys Gly | Cys Arg Trp Gly Asp | Gly | | |
| | | 445 | | 450 | | 455 |
| | Gln Arg Asn Asp Gly | Leu Leu Phe *** | Gly Val Ser Glu Arg | Leu | | |
| | | 460 | | 465 | | 470 |
| 35 | Val Tyr Ala Leu Leu | Thr Asp Pro Met | Leu Asp Leu Ser Pro | Phe | | |
| | | 475 | | 480 | | 485 |
| | Leu Leu Ser Phe Phe | Ser Ser His Leu | Pro His Ser Thr Leu | Pro | | |
| | | 490 | | 495 | | 500 |
| | Ser Trp Asp Leu Pro | Ser Leu Ser Arg | Gln Pro Ser Ala Met | Ala | | |
| | | 505 | | 510 | | 515 |
| 40 | Leu Pro Val Pro Pro | Ser Pro Phe Phe | Gln Gly Ala Glu Arg | Trp | | |
| | | 520 | | 525 | | 530 |
| | Pro Pro Gly Val Ala | Leu Ser Tyr Leu | His Ser Leu Pro Leu | Lys | | |
| | | 535 | | 540 | | 545 |
| 45 | Met Gly Gly Asp Gln | Arg Ser Met Gly | Leu Ala Cys Glu Ser | Pro | | |
| | | 550 | | 555 | | 560 |
| | Leu Ala Ala Trp Ser | Leu Gly Ile Thr | Pro Ala Leu Val Leu | Gln | | |
| | | 565 | | 570 | | 575 |
| | Met Leu Leu Gly Phe | Ile Gly Ala Gly | Pro Ser Arg Ala Gly | Pro | | |
| | | 580 | | 585 | | 590 |
| 50 | Leu Thr Leu Pro Ala | Trp Leu His Ser | Pro *** Arg Leu Pro | Leu | | |
| | | 595 | | 600 | | 605 |
| | Val His Pro Phe Ile | Glu Arg Pro Ala | Leu Leu Gln Ser Ser | Gly | | |
| | | 610 | | 615 | | 620 |
| 55 | Leu Pro Pro Ala Ala | Arg Leu Ser Thr | Arg Gly Leu Ser *** | Asp | | |
| | | 625 | | 630 | | 635 |
| | Val Gln Gly Pro Arg | Pro Ala Gly Thr | Ala Ser Pro Asn Leu | Gly | | |
| | | 640 | | 645 | | 650 |
| | Pro Trp Lys Ser Pro | Pro Pro His His | *** Ser Ala Leu Thr | Leu | | |
| | | 655 | | 660 | | 665 |
| 60 | Gly Phe His Gly Pro | His Ser Thr Ala | Ser Pro Thr *** | Ala | | |
| | | 670 | | 675 | | 680 |
| | Asp Leu Gly Thr Lys | Gly Gly Val Pro | Arg Leu Leu *** | Leu | | |
| | | | | Ser | | |

685 690 695
 Arg Gly Ser Gly His Val Gln Gly Gly Ala Gly Trp Pro Gly Gly
 700 705 710
 5 Ser Ala His Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys
 715 720 725
 Ile Leu Glu Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala
 730 735 740
 Gly Gln Cys Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val
 745 750 755
 10 Gly Pro Xxx

(2) INFORMATION FOR SEQ ID NO:45:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 746 nucleic acids
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

25 CGTATGTCAC TCCATTCCAA ACTCGTTCAT GGTATCATAA ATATCAACAC ATTTACGCTC 60
 CACTCCTCTA TGGTATTTAC ACACTCAAAT ATCGTACTCA AGATTGGGAA GCTTTTGTA 120
 AGGATGGTAA AAATGGTGCA ATTCGTGTGA GTGTCGCCAC AAATTCGAT AAGGCCGCTT 180
 ACGTCATTGG TAAATTGTCT TTTGTTTTCT TCCGTTTCAT CCTTCCACTC CGTTATCATA 240
 30 GCTTTACAGA TTTAATTTGT TATTTCTCA TTGCTGAATT CGTCTTTGGT TGGTATCTCA 300
 CAATTAATTT CCAAGTTAGT CATGTCGCTG AAGATCTCAA ATTCTTTGCT ACCCCTGAAA 360
 GACCAGATGA ACCATCTCAA ATCAATGAAG ATGGGGCAAT CCTTCAACTT AAAACTACTC 420
 AAGATTATGG TCATGGTTCA CTCCTTTGTA CCTTTTTTAG TGGTCTTTA AATCATCAAG 480
 TTGTTTCATCA TTTATTCCCA TCAATTGCTC AAGATTTCTA CCCACAACCT GTACCAATTG 540
 TAAAAGAAGT TTGTAAAGAA CATAACATTA CTTACCACAT TAAACCAAAC TTCCTGAAG 600
 35 CTATTATGTC ACACATTAAT TACCTTTACA AAATGGGTAA TGATCCAGAT TATGTTAAAA 660
 AACCATTAGC CTCAAAAGAT GATTAAATGA AATAACTTAA AAACCAATTA TTTACTTTTG 720
 ACAAACAGTA ATATTAATAA ATACAA 746

40 (2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 227 amino acids
 45 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Tyr Val Thr Pro Phe Gln Thr Arg Ser Trp Tyr His Lys Tyr Gln
 1 5 10 15
 55 His Ile Tyr Ala Pro Leu Leu Tyr Gly Ile Tyr Thr Leu Lys Tyr
 20 25 30
 Arg Thr Gln Asp Trp Glu Ala Phe Val Lys Asp Gly Lys Asn Gly
 35 40 45
 Ala Ile Arg Val Ser Val Ala Thr Asn Phe Asp Lys Ala Ala Tyr
 50 55 60
 60 Val Ile Gly Lys Leu Ser Phe Val Phe Phe Arg Phe Ile L u Pro
 65 70 75
 Leu Arg Tyr His Ser Phe Thr Asp Leu Ile Cys Tyr Phe Leu Ile
 80 85 90
 65 Ala Glu Phe Val Phe Gly Trp Tyr Leu Thr Ile Asn Phe Gln Val
 95 100 105

Ser His Val Ala Glu Asp Leu Lys Phe Phe Ala Thr Pro Glu Arg
 110 115 120
 Pro Asp Glu Pro Ser Gln Ile Asn Glu Asp Trp Ala Ile Leu Gln
 125 130 135
 5 Leu Lys Thr Thr Gln Asp Tyr Gly His Gly Ser Leu Leu Cys Thr
 140 145 150
 Phe Phe Ser Gly Ser Leu Asn His Gln Val Val His His Leu Phe
 155 160 165
 10 Pro Ser Ile Ala Gln Asp Phe Tyr Pro Gln Leu Val Pro Ile Val
 170 175 180
 Lys Glu Val Cys Lys Glu His Asn Ile Thr Tyr His Ile Lys Pro
 185 190 195
 Asn Phe Thr Glu Ala Ile Met Ser His Ile Asn Tyr Leu Tyr Lys
 200 205 210
 15 Met Gly Asn Asp Pro Asp Tyr Val Lys Lys Pro Leu Ala Ser Lys
 215 220 225
 Asp Asp ***

(2) INFORMATION FOR SEQ ID NO 47:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 494 nucleic acids
 (B) TYPE: nucleic acid
 25 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTTTGAAGG NTCCAAGTTN ACCACGGANT NGGCAAGTTN ACGGGGCGGA AANCGGTTTT 60
 CCCCCAAGC CTTTGTGCGA CTGGTTCTGT GGTGGCTTCC AGTACCAAGT CGACCACCAC 120
 35 TTATTCGCCA GCCTGCCCCG ACACAATCTG GCCAAGACAC ACGCACTGGT CGAATCGTTC 180
 TGCAAGGAGT GGGGTGTCCA GTACCACGAA GCCGACCTCG TGGACGGGAC CATGGAAGTC 240
 TTGCACCATT TGGGCAGCGT GGCCGGCGAA TTCGTCGTGG ATTTGTACG CGACGGACCC 300
 GCCATGTAAT CGTCGTTCGT GACGATGCAA GGGTTCACGC ACATCTACAC AACTCACTC 360
 ACACAAC TAGTAACTCGT ATAGAATTCTG GTGTCGACCT GGACCTTGTT TGA CTGGTTG 420
 40 GGGATAGGGT AGGTAGGCGG ACGCGTGGGT CGNCCCCGGG AATTCTGTGA CCGGTACCTG 480
 GCCCGCGTNA AAGT 494

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 87 amino acids
 (B) TYPE: amino acid
 50 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Phe Trp Lys Xxx Pro Ser Xxx Pro Arg Xxx Xxx Gln Val Xxx Gly
 1 5 10 15
 60 Ala Glu Xxx Gly Phe Pro Pro Lys Pro Phe Val Asp Trp Phe Cys
 20 25 30
 Gly Gly Phe Gln Tyr Gln Val Asp His His Leu Phe Pro Ser Leu
 35 40 45
 Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val Glu Ser Phe
 50 55 60
 65 Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu Val Asp
 65 70 75

Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly Glu
 65 70 75
 Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
 80 85

5

10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 520 nucleic acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

25

```

GGATGGAGTT CGTCTGGATC GCTGTGCGCT ACGCGACGTG GTTTAAGCGT CATGGGTGCG      60
CTTGGGTACA CGCCGGGGCA GTCGTTGGGC ATGTAATTGT GCGCCTTTGG TCTCGGCTGC      120
ATTACATTTT TTCTGCAGTT CGCCGTAAGT CACACCCATT TGCCCGTGAG CAACCCGGAG      180
GATCAGCTGC ATTGGCTCGA GTACGCGCGG ACCACACTGT GAACATCAGC ACCAAGTCGT      240
GGTTTGTCAC ATGGTGGATG TCGAACCTCA ACTTTCAGAT CGAGCACCAC CTTTTCCCCA      300
CGGCGCCCCA GTTCCGTTTC AAGGAGATCA GCCCGCGCGT CGAGGCCCTC TTCAAGCGCC      360
ACGGTCTCCC TTACTACGAC ATGCCCTACA CGAGCGCCGT CTCCACCACC TTTGCCAACC      420
TCTACTCCGT CGGCCATTCC GTCGGCGACG CCAAGCGCGA CTAGCCTCTT TTCCTAGACC      480
TTAATTCCCC ACCCCACCCC ATGTTCTGTC TTCCTCCGCG      520

```

35

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH: 153 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

50

```

Met Glu Phe Val Trp Ile Ala Val Arg Tyr Ala Thr Trp Phe Lys
1           5           10           15
Arg His Gly Cys Ala Trp Val His Ala Gly Ala Val Val Gly His
20           25           30
Val Leu Val Arg Leu Trp Ser Arg Leu His Leu His Phe Ser Ala
35           40           45
Val Arg Arg Lys Ser His Pro Phe Ala Arg Glu Gln Pro Gly Gly
55           60           65
Ser Ala Ala Leu Ala Arg Val Arg Ala Asp His Thr Val Asn Ile
65           70           75
Ser Thr Lys Ser Trp Phe Val Thr Trp Trp Met Ser Asn Leu Asn
80           85           90
Phe Gln Ile Glu His His Leu Phe Pro Thr Ala Pro Gln Phe Arg
95           100          105
Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu Phe Lys Arg His
110          115          120
Gly Leu Pro Tyr Tyr Asp Met Pro Tyr Thr Ser Ala Val Ser Thr
125          130          135
Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly Asp Ala

```

65

| | | | |
|-------------|-----|-----|-----|
| | 140 | 145 | 150 |
| Lys Arg Asp | | | |

5

(2) INFORMATION FOR SEQ ID NO:51:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 429 nucleic acids
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

20

| | | | | | | |
|------------|------------|-------------|------------|-------------|-------------|-----|
| ACGCGTCCGC | CCACGCGTCC | GCCGCGAGCA | ACTCATCAAG | GAAGGCTACT | TTGACCCCTC | 60 |
| GCTCCCGCAC | ATGACGTACC | GCGTGGTCTGA | GATTGTTGTT | CTCTTCGTGC | TTTCCTTTTG | 120 |
| GCTGATGGGT | CAGTCTTAC | CCCTCGCGCT | CGCTCTCGGC | ATTGTCGTCA | GCGGCATCTC | 180 |
| TCAGGGTTCG | TGCGGCTGGG | TAATGCATGA | GATGGGCCAT | GGGTGCTTCA | CTGGTGTCTAT | 240 |
| TTGGCTTGAC | GACCGGTTGT | GCGAGTCTTT | TTACGGCGCT | GTTTGTGGCA | TGAGCGGTCA | 300 |
| TTACTGGAAA | AACCAGCACA | GCAAAACCCA | CGCAGCGCCA | AACCGGCTCG | AGCACGATGT | 360 |
| AGATCTCAAC | ACCTTGCCAT | TGGTGGCCTT | CAACAGCGCG | GTCGCTGCGCA | AGGTCCGACC | 420 |

30

(2) INFORMATION FOR SEQ ID NO:52:

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

45

[illegible]

50

55

60

What is claimed is:

1. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, wherein said one or more nucleotide sequences is linked to a heterologous nucleotide sequence.

2. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, wherein said one or more nucleotide sequences is operably associated with an expression control sequence functional in a plant cell.

3. The nucleic acid construct according to claim 2, wherein said nucleotide sequence has an average A + T content of less than about 60%.

4. The nucleic acid construct according to claim 2, wherein said nucleotide sequence is derived from a fungus.

5. The nucleic acid construct according to claim 4, wherein said fungus is of the genus *Mortierella*.

6. The nucleic acid construct according to claim 5, wherein said fungus is of the species *alpina*.

7. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:2, wherein said nucleotide sequence is

operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 6 from the carboxyl end of said fatty acid molecule.

5

8. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:4, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence functional in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 12 from the carboxyl end of said fatty acid molecule.

10

9. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 5 from the carboxyl end of said fatty acid molecule.

15

20

10. A nucleic acid construct comprising:

at least one nucleotide sequence which encodes a functionally active desaturase having an amino acid sequence depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a promoter functional in a plant cell.

25

11. The nucleic acid construct according to claim 10, wherein said plant cell is a seed cell.

5 12. The nucleic acid construct according to claim 11, wherein said seed cell is an embryo cell.

13. A recombinant plant cell comprising:

10 At least one copy of a DNA sequence which encodes at least one functionally active *Mortierella alpina* fatty acid desaturase which results in the production of a polyunsaturated fatty acid, wherein said fatty acid desaturase has an amino acid sequence as depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, wherein said cell was transformed with a vector comprising said DNA sequence, and wherein said DNA sequence is operably associated with an expression control
15 sequence.

14. The recombinant plant cell of claim 13, wherein said polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

20

15. The recombinant plant cell of claim 13, wherein said recombinant plant cell is enriched in a fatty acid selected from the group consisting of 18:1, 18:2, 18:3 and 18:4.

25 16. The recombinant plant cell of claim 15, wherein said plant cell is selected from the group consisting of *Brassica*, soybean, safflower, corn, flax, and sunflower.

17. The recombinant plant cell according to claim 16, wherein said expression control sequence is endogenous to said plant cell.

18. One or more plant oils expressed by said recombinant plant cell of claim 16.

5

19. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain a transgene encoding a transgene expression product which desaturates a fatty acid molecule at carbon 5 from the carboxyl end of said fatty acid molecule, wherein said transgene is operably associated with an expression control sequence, under conditions whereby said transgene is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

20. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain one or more transgenes, derived from a fungus or algae, which encodes a transgene expression product which desaturates a fatty acid molecule at a carbon selected from the group consisting of carbon 5, carbon 6 and carbon 12 from the carboxyl end of said fatty acid molecule, wherein said one or more transgenes is operably associated with an expression control sequence, under conditions whereby said one or more transgenes is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

25

21. The method according to claims 19 or 20, wherein said long chain polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

22. A plant oil or fraction thereof produced according to the method of claims 19 or 20.
23. A method of treating or preventing malnutrition comprising administering
5 said plant oil of claim 22 to a patient in need of said treatment or prevention in an amount sufficient to effect said treatment or prevention.
24. A pharmaceutical composition comprising said plant oil or fraction of claim 22 and a pharmaceutically acceptable carrier.
- 10 25. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is in the form of a solid or a liquid.
- 15 26. The pharmaceutical composition of claim 25, wherein said pharmaceutical composition is in a capsule or tablet form.
- 20 27. The pharmaceutical composition of claim 24 further comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.
28. A nutritional formula comprising said plant oil or fraction thereof of claim 22.
- 25 29. The nutritional formula of claim 28, wherein said nutritional formula is selected from the group consisting of an infant formula, a dietary supplement, and a dietary substitute.

30. The nutritional formula of claim 29, wherein said infant formula, dietary supplement or dietary supplement is in the form of a liquid or a solid.

31. An infant formula comprising said plant oil or fraction thereof of claim 22.

5

32. The infant formula of claim 31 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electro dialysed whey, electro dialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

10

33. The infant formula of claim 32 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

15

34. A dietary supplement comprising said plant oil or fraction thereof of claim 22.

20

35. The dietary supplement of claim 34 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electro dialysed whey, electro dialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

25

36. The dietary supplement of claim 35 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium,

magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

5 37. The dietary supplement of claim 34 or claim 36, wherein said dietary supplement is administered to a human or an animal.

38. A dietary substitute comprising said plant oil or fraction thereof of claim 22.

10 39. The dietary substitute of claim 38 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

15 40. The dietary substitute of claim 39 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

20 41. The dietary substitute of claim 38 or claim 40, wherein said dietary substitute is administered to a human or animal.

25 42. A method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to said patient said dietary substitute of claim 38 or said dietary supplement of claim 34 in an amount sufficient to effect said treatment.

43. The method of claim 42, wherein said dietary substitute or said dietary supplement is administered enterally or parenterally.

44. A cosmetic comprising said plant oil or fraction thereof of claim 22.

5

45. The cosmetic of claim 44, wherein said cosmetic is applied topically.

46. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is administered to a human or an animal.

10

47. An animal feed comprising said plant oil or fraction thereof of claim 22.

48. An isolated nucleotide sequence comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:38 - SEQ ID NO:44 wherein said nucleotide sequence is expressed in a plant cell.

15

49. The method of claim 20 wherein said fungus is *Mortierella species*.

50. The method of claim 49 wherein said fungus is *Mortierella alpina*.

20

51. An isolated nucleotide sequence selected from the group consisting of SEQ ID NO:49 - SEQ ID NO:50 wherein said sequence is expressed in a plant cell.

1/20

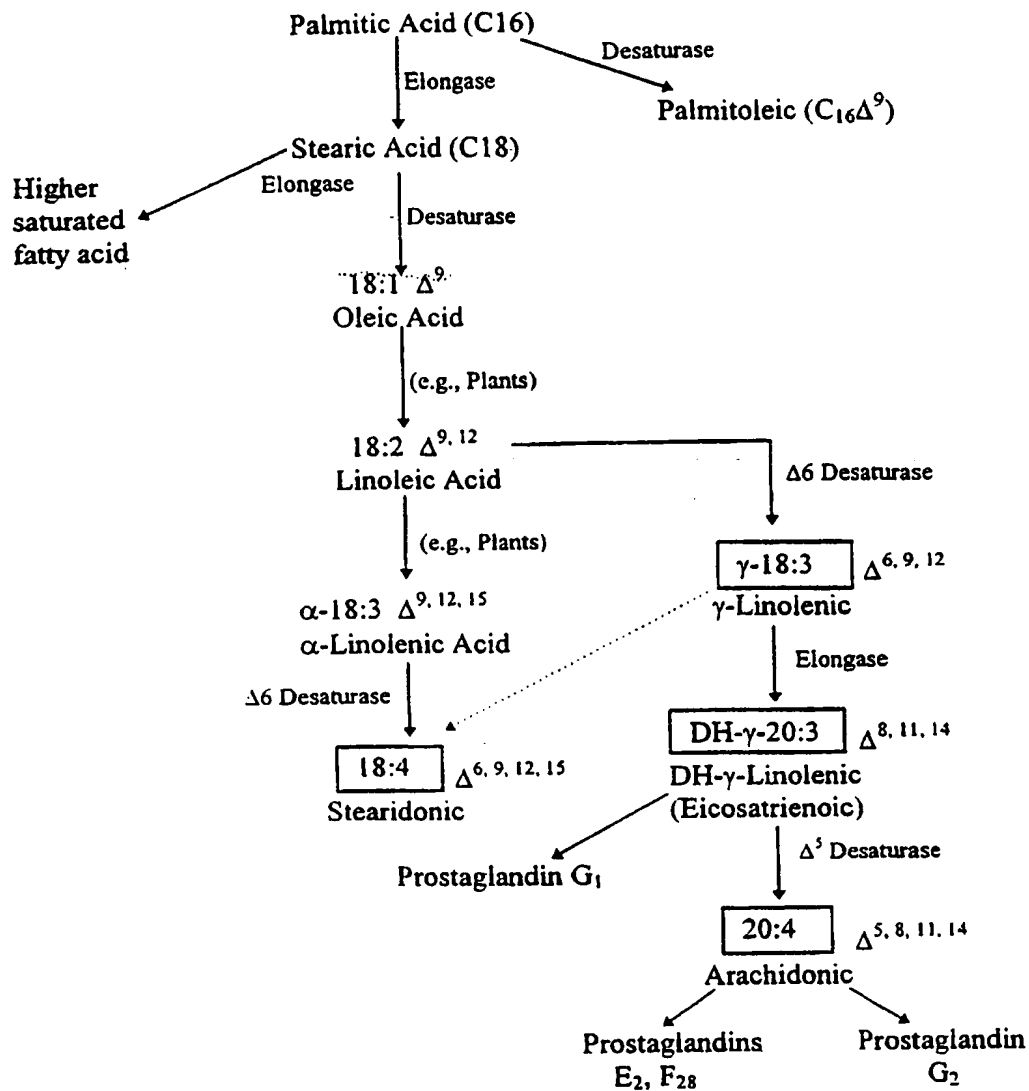
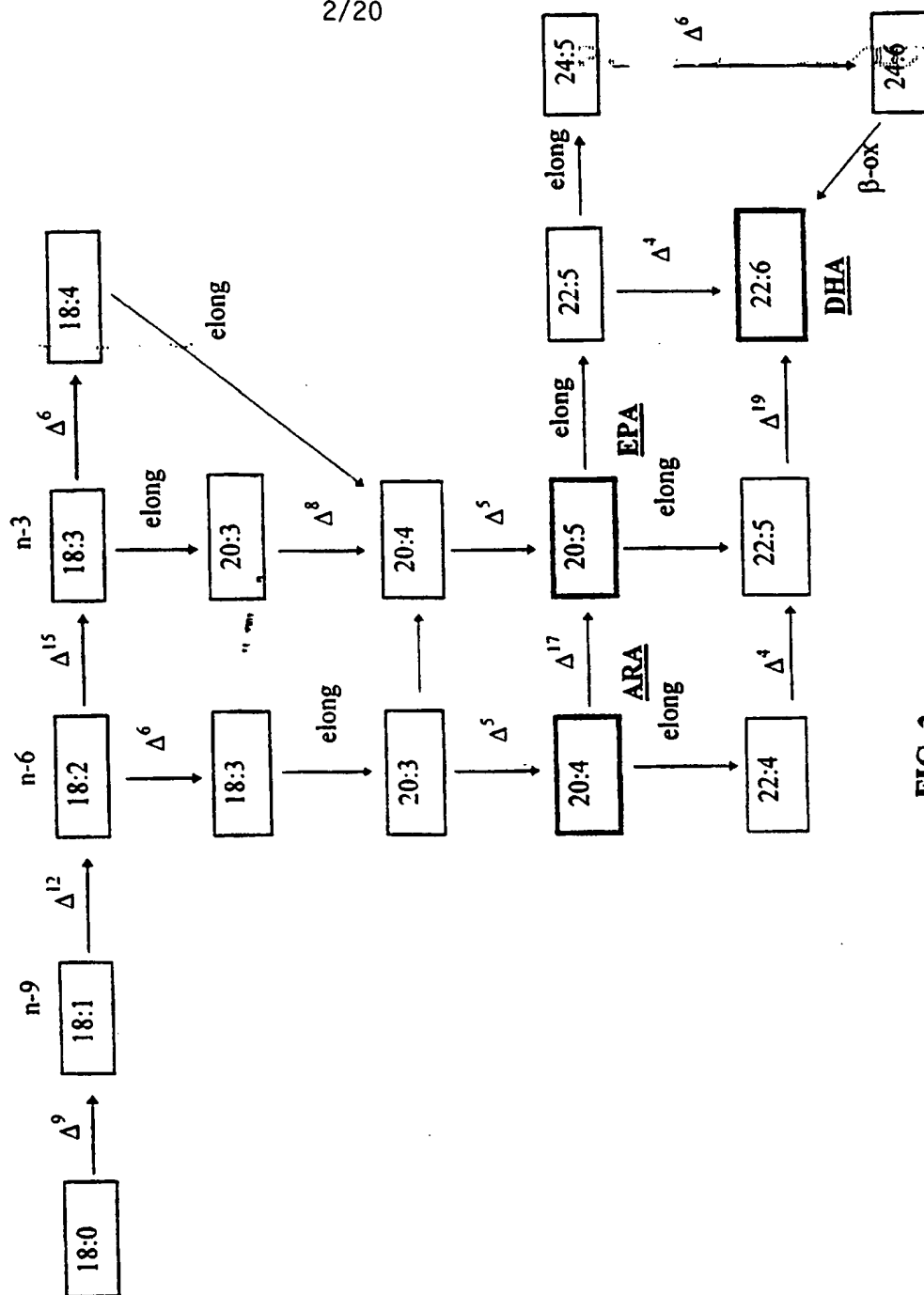


FIG. 1

PUFA PATHWAYS**FIG. 2**

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60 *
CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCCTTC AACCCCCCTC TTTGACAAAG
ACAAACAAACC ATG GCT GCT CCC AGT GTG AGG ACG TTT ACT GGG CCC GAG
Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu

120 *
GTT TTG AAT GCC GAG GCT CTG AAT GAG GGC AAG AAG GAT GCC GAG GCA
Val Leu Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala

180 *
CCC TTC TTG ATG ATC ATC GAC AAC AAG GTG TAC GAT GTC CGC GAG TTC
Pro Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe

240 *
GTC CCT GAT CAT CCC GGT GGA AGT GTG ATT CTC ACG CAC GTT GGC AAG
Val Pro Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys

300 *
GAC GGC ACT GAC GTC TTT GAC ACT TTT CAC CCC GAG GCT GCT TGG GAG
Asp Gly Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu

ACT CTT GCC AAC TTT TAC GTT GGT GAT ATT GAC GAG AGC GAC CGC GAT
Thr Leu Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp

360 *
ATC AAG AAT GAT GAC TTT GCG GCC GAG GTC CGC AAG CTG CGT ACC TTG
Ile Lys Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu

```

FIG. 3A

420 *
 TTC CAG TCT CTT GGT TAC TAC GAT TCT TCC AAG GCA TAC TAC GCC TTC
 Phe Gln Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe
 480 *
 AAG GTC TCG TTC AAC CTC TGC ATC TGG GGT TTG TCG ACG GTC ATT GTG
 Lys Val Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val
 540 *
 GCC AAG TGG GGC CAG ACC TCG ACC CTC GCC AAC GTG CTC TCG GCT GCG
 Ala Lys Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala
 CTT TTG GGT CTG TTC TGG CAG CAG TGC GGA TGG TTG GCT CAC GAC TTT
 Leu Leu Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe
 600 *
 TTG CAT CAC CAG GTC TTC CAG GAC CAG CGT TTC TGG GGT GAT CTT TTC GGC
 Leu His His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly
 660 *
 GCC TTC TTG GGA GGT GTC TGC CAG GGC TTC TCG TCC TCG TGG TGG AAG
 Ala Phe Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys
 720 *
 GAC AAG CAC AAC ACT CAC CAC GCC GCC CCC AAC GTC CAC GGC GAG GAT
 Asp Lys His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp
 780 *

FIG. 3B

5/20

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CCC GAC ATT GAC ACC CAC CCT CTG TTG ACC TGG AGT GAG CAG GCG TTG
Pro Asp Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu

GAG ATG TTC TCG GAT GTC CCA GAT GAG GAG CTG ACC CGC ATG TGG TCG
Glu Met Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser

      840 *
CGT TTC ATG GTC CTG AAC CAG ACC TGG TTT TAC TTC CCC ATT CTC TCG
Arg Phe Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser

      900 *
TTT GCC CGT CTC TCC TGG TGC CTC CAG TCC ATT CTC TTT GTG CTG CCT
Phe Ala Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro

      960 *
AAC GGT CAG GCC CAC AAG CCC TCG GGC GCG CGT GTG CCC ATC TCG TTG
Asn Gly Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu

      1020 *
GTC GAG CAG CTG TCG CTT GCG ATG CAC TGG ACC TGG TAC CTC GCC ACC
Val Glu Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr

ATG TTC CTG TTC ATC AAG GAT CCC GTC AAC ATG CTG GTG TAC TTT TTG
Met Phe Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu

      1080 *
GTG TCG CAG GCG GTG TGC GGA AAC TTG TTG GCG ATC GTG TTC TCG CTC
Val Ser Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu

```

FIG. 3C

6/20

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1140 *
AAC CAC AAC GGT ATG CCT GTG ATC TCG AAG GAG GAG GCG GTC GAT ATG
Asn His Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met

1200 *
GAT TTC TTC ACG AAG CAG ATC ATC ACG GGT CGT GAT GTC CAC CCG GGT
Asp Phe Phe Thr Lys Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly

1260 *
CTA TTT GCC AAC TGG TTC ACG GGT GGA TTG AAC TAT CAG ATC GAG CAC
Leu Phe Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His

1320 *
CAC TTG TTC CCT TCG ATG CCT CGC CAC AAC TTT TCA AAG ATC CAG CCT
His Leu Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro

1380 *
GCT GTC GAG ACC CTG TGC AAA AAG TAC AAT GTC CGA TAC CAC ACC ACC
Ala Val Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr

1440 *
GGT ATG ATC GAG GGA ACT GCA GAG GTC TTT AGC CGT CTG AAC GAG GTC
Gly Met Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val

TCC AAG GCT GCC TCC AAG ATG GGT AAG GCG CAG TAAAAAAA AAACAAGGAC
Ser Lys Ala Ala Ser Lys Met Gly Lys Ala Gln

```

FIG. 3D

1500 *
GTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT TGTCAAGTCG AGCGTTTCTG
1560 *
GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC CCCCCGCTCA TATCTCATTC
ATTTCCTCTTA TTAAACAACCT TGTCCCCCCC TTCACCG

FIG. 3E

FIG. 4

FIG. 1

9/20

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60 *
GTCCCTGTC GCTGTGGCA CACCCCATCC TCCCTCGCTC CCTCTGGTT TGTCTTGGC
120 *
CCACCGTATC TCTTCCACC TCGAGACGA CTGCACTGT AATCAGGAAC CGACAAAPAC
180 *
ACGATTTCCTT TTTACTCAGC ACCAACTCAA AATCTCAAC CGCAACCCCTT TTTACGG ATG
Met
GCA CCT CCC AAC ACT ATC GAT GCC GGT TTG ACC CAG CGT CAT ATC AGC
Ala Pro Pro Asn Thr Ile Asp Ala Gly Leu Thr Gln Arg His Ile Ser
240 *
ACC TCG GCC CCA AAC TCG GCC AAG CCT GCC TTC GAG CGC AAC TAC CAG
Thr Ser Ala Pro Asn Ser Ala Lys Pro Ala Phe Glu Arg Asn Tyr Gln
300 *
CTC CCC GAG TTC ACC ATC AAG GAG ATC CGA GAG TGC ATC CCT GCC CAC
Leu Pro Glu Phe Thr Ile Lys Glu Ile Arg Glu Cys Ile Pro Ala His
360 *
TGC TTT GAG CGC TCC GGT CTC CGT GGT CTC TGC CAC GTT GCC ATC GAT
Cys Phe Glu Arg Ser Gly Leu Arg Gly Leu Cys His Val Ala Ile Asp
420 *
CTG ACT TGG GCG TCG CTC TTG TTC CTG GCT GCG ACC CAG ATC GAC AAG
Leu Thr Trp Ala Ser Leu Leu Phe Leu Ala Ala Thr Gln Ile Asp Lys
TTT GAG AAT CCC TTG ATC CGC TAT TTG GCC TGG CCT GTT TAC TGG ATC
Phe Glu Asn Pro Leu Ile Arg Tyr Leu Ala Trp Pro Val Tyr Trp Ile

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FIG. 5A

10/20

480
 ATG CAG GGT ATT GTC TGC ACC GGT GTC TGG GTG CTG GCT CAC GAG TGT
 Met Gln Gly Ile Val Cys Thr Gly Val Trp Val Leu Ala His Glu Cys

540
 GGT CAT CAG TCC TTC TCG ACC TCC AAG ACC CTC AAC ACA ACA GTT GGT
 Gly His Gln Ser Phe Ser Thr Ser Lys Thr Leu Asn Thr Val Gly

600
 TGG ATC TTG CAC TCG ATG CTC TTG GTC CCC TAC CAC TCC TGG AGA ATC
 Trp Ile Leu His Ser Met Leu Leu Val Pro Tyr His Ser Trp Arg Ile

660
 TCG CAC TCG AAG CAC CAC AAG GCC ACT GGC CAT ATG ACC AAG GAC CAG
 Ser His Ser Lys His His Lys Ala Thr Gly His Met Thr Lys Asp Gln

720
 GTC TTT GTG CCC AAG ACC CGC TCC CAG GTT GGC TTG CCT CCC AAG GAG
 Val Phe Val Pro Lys Thr Arg Ser Gln Val Gly Leu Pro Pro Lys Glu

780
 AAC GCT GCT GCT GCC GTT CAG GAG GAG GAC ATG TCC GTG CAC CTG GAT
 Asn Ala Ala Ala Ala Val Gln Gln Glu Asp Met Ser Val His Leu Asp

840
 GAG GAG GCT CCC ATT GTG ACT TTG TTC TCG ATG GTG ATC CAG TTC TTG
 Glu Glu Ala Pro Ile Val Thr Leu Phe Trp Met Val Ile Gln Phe Leu

840
 TTC GGA TGG CCC GCG TAC CTG ATT ATG AAC GCC TCT GGC CAA GAC TAC
 Phe Gly Trp Pro Ala Tyr Leu Ile Met Asn Ala Ser Gly Gln Asp Tyr

FIG. 5B

11/20

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990 *
GGC CGC TGG ACC TCG CAC TTC CAC ACG TAC TCG CCC ATC TTT GAG CCC
Gly Arg Trp Thr Ser His Phe His Thr Tyr Ser Pro Ile Phe Glu Pro

CGC AAC TTT TTC GAC ATT ATT ATC TCG GAC CTC GGT GTG TTG GCT GCC
Arg Asn Phe Phe Asp Ile Ile Ile Ser Asp Leu Gly Val Leu Ala Ala

960 *
CTC GGT GCC CTG ATC TAT GCC TCC ATG CAG TTG TCG CTC TTG ACC GTC
Leu Gly Ala Leu Ile Tyr Ala Ser Met Gln Leu Ser Leu Leu Thr Val

1020 *
ACC AAG TAC TAT ATT GTC CCC TAC CTC TTT GTC AAC TTT TGG TTG GTC
Thr Lys Tyr Tyr Ile Val Pro Tyr Leu Phe Val Asn Phe Trp Leu Val

1080 *
CTG ATC ACC TTC TTG CAG CAC ACC GAT CCC AAG CTG CCC CAT TAC CGC
Leu Ile Thr Phe Leu Gln His Thr Asp Pro Lys Leu Pro His Tyr Arg

1140 *
GAG GGT GCC TGG AAT TTC CAG CGT GGA GCT CTT TGC ACC GTT GAC CGC
Glu Gly Ala Trp Asn Phe Gln Arg Gly Ala Leu Cys Thr Val Asp Arg

TCG TTT GGC AAG TTC TTG GAC CAT ATG TTC CAC GGC ATT GTC CAC ACC
Ser Phe Gly Lys Phe Leu Asp His Met Phe His Gly Ile Val His Thr

1200 *
CAT GTG GCC CAT CAC TTG TTC TCG CAA ATG CCG TTC TAC CAT GCT GAG
His Val Ala Ala His His Leu Phe Ser Gln Met Pro Phe Tyr His Ala Glu

```

FIG. 5C

12/20

1260
GAA GCT ACC TAT CAT CTC AAG AAA CTG CTG GGA GAG TAC TAT GTG TAC
Glu Ala Thr Tyr His Leu Lys Lys Leu Leu Gly Glu Tyr Tyr Val Tyr
1320
GAC CCA TCC CCG ATC GTC GTT GCG GTC TGG AGG TCG TTC CGT GAG TGC
ASP Pro Ser Pro Ile Val Val Ala Val Trp Arg Ser Phe Arg Glu Cys
1380
CGA TTC GTG GAG GAT CAG GGA GAC GTG GTC TTT TTT AAG AAG TAAAAA
Arg Phe Val Glu Asp Gln Gly Asp Val Val Phe Phe Lys Lys
1440
AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC
CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTGATTC GCGCCCTCC

FIG. 5D

13/20

FIG. 6

| | | | | | |
|------------|------------|------------|------------|------------|------------|
| 10 | 20 | 30 | 40 | 50 | 60 |
| | | | | | * |
| LHHTYTNIAG | ADPDVSTSEP | DVRRIKPNQK | WVFNHINQHM | FVPFLYGLLA | FKVRIQDINI |
| 70 | 80 | 90 | 100 | 110 | 120 |
| | | | | | * |
| LYFVKTNDAI | RVNPISTWHT | VMFWGGKAFF | VWYRLIVPLQ | YLPLGKVLLL | FTVADMVSSY |
| 130 | 140 | 150 | 160 | 170 | 180 |
| | | | | | * |
| WLALTFQANY | VVEEVQWPLP | DENGIIQKDW | AAMQVETTQD | YAHDSHLWTS | ITGSLNYQXV |
| HHLFPH | | | | | |

14/20

FIG. 7A

GCTTCTCTCCA GTTCATCCTC CATTTCGCCA CCTGCATTCT TTACGACCGT TAAGCAAG
 60 *
 ATG GGA ACG GAC CAA GGA AAA ACC TTC ACC TGG GAA GAG CTG GCG GCC
 Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala
 120 *
 CAT AAC ACC AAG GAC GAC CTA CTC TTG GCC ATC CGC GGC AGG GTG TAC
 His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr
 180 *
 GAT GTC ACA AAG TTC TTG AGC CGC CAT CCT GGT GGA GTG GAC ACT CTC
 Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu
 240 *
 CTG CTC GGA GCT GGC CGA GAT GTT ACT CCG GTC TTT GAG ATG TAT CAC
 Leu Leu Gly Ala Gly Arg Asp Val Thr Pro Val Phe Glu Met Tyr His
 GCG TTT GGG GCT GCA GAT GCC ATT ATG AAG AAG TAC TAT GTC GGT ACA
 Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr
 300 *
 CTG GTC TCG AAT GAG CTG CCC ATC TTC CCG GAG CCA ACG GTG TTC CAC
 Leu Val Ser Asn Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His
 360 *
 AAA ACC ATC AAG ACG AGA GTC GAG GGC TAC TTT ACG GAT CGG AAC ATT
 Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile

FIG. 7B

```

      420 *
GAT CCC AAG AAT AGA CCA GAG ATC TGG GGA CGA TAC GCT CTT ATC TTT
Asp Pro Lys Asn Arg Pro Glu Ile Trp Gly Arg Tyr Ala Leu Ile Phe

      480 *
GGA TCC TTG ATC GCT TCC TAC TAC GCG CAG CTC TTT GTG CCT TTC GTT
Gly Ser Leu Ile Ala Ser Tyr Tyr Ala Gln Leu Phe Val Pro Phe Val

GTC GAA CGC ACA TGG CTT CAG GTG GTG TTT GCA ATC ATC ATG GGA TTT
Val Glu Arg Thr Trp Leu Gln Val val Phe Ala Ile Ile Met Gly Phe

540 *
GCG TGC GCA CAA GTC GGA CTC AAC CCT CTT CAT GAT GCG TCT CAC TTT
Ala Cys Ala Gln Val Gly Leu Asn Pro Leu His Asp Ala Ser His Phe

      600 *
TCA GTG ACC CAC AAC CCC ACT CTC TGG AAG ATT CTG GGA GCC ACG CAC
Ser Val Thr His Asn Pro Thr Val Trp Lys Ile Leu Gly Ala Thr His

      660 *
GAC TTT TTC AAC GGA GCA TCG TAC CTG GTG TGG ATG TAC CAA CAT ATG
Asp Phe Phe Asn Gly Ala Ser Tyr Leu Val Trp Met Tyr Gln His Met

      720 *
CTC GGC CAT CAC CCC TAC ACC AAC ATT GCT GGA GCA GAT CCC GAC GTG
Leu Gly His His Pro Tyr Thr Asn Ile Ala Gly Ala Asp Pro Asp Val

```

FIG. 7C

```

TCG ACG TCT GAG CCC GAT GTT CGT CGT ATC AAG CCC AAC CAA AAG TGG
Ser Thr Ser Glu Pro Asp Val Arg Arg Ile Lys Pro Asn Gln Lys Trp
780 *
TTT GTC AAC CAC ATC AAC CAG CAC ATG TTT GTT CCT TTC CTG TAC GGA
Phe Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly
840 *
CTG CTG GCG TTC AAG GTG CGC ATT CAG GAC ATC AAC ATT TTG TAC TTT.
Leu Leu Ala Phe Lys Val Arg Ile Gln Asp Ile Asn Ile Leu Tyr Phe
900 *
GTC AAG ACC AAT GAC GCT ATT CGT GTC AAT CCC ATC TCG ACA TGG CAC
Val Lys Thr Asn Asp Ala Ile Arg Val Asn Pro Ile Ser Thr Trp His
960 *
ACT GTG ATG TTC TGG GGC GGC AAG GCT TTC TTT GTC TGG TAT CGC CTG
Thr Val Met Phe Trp Gly Gly Lys Ala Phe Phe Val Trp Tyr Arg Leu
ATT GTT CCC CTG CAG TAT CTG CCC CTG GGC AAG GTG CTG CTC TTG TTC
Ile Val Pro Leu Gln Tyr Leu Pro Leu Gly Lys Val Leu Leu Phe
1020 *
ACG GTC GCG GAC ATG GTG TCG TCT TAC TGG CTG GCG CTG ACC TTC CAG
Thr Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln

```

FIG. 7D

1080
GCG AAC CAC GTT GTT GAG GAA GTT CAG TGG CCG TTG CCT GAC GAG AAC
Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn

1140
GGG ATC ATC CAA AAG GAC TGG GCA GCT ATG CAG GTC GAG ACT ACG CAG
Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln

1200
GAT TAC GCA CAC GAT TCG CAC CTC TGG ACC AGC ATC ACT GGC AGC TTG
Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu

1260
AAC TAC CAG GCT GTG CAC CAT CTG TTC CCG AAC GTG TCG CAG CAC CAT
Asn Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His

1320
TAT CCC GAT ATT CTG GCC ATC ATC AAG AAC ACC TGC AGC GAG TAC AAG
Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys

1380
GTT CCA TAC CTT GTC AAG GAT ACG TTT TGG CAA GCA TTT GCT TCA CAT
Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His

1440
TTG GAG CAC TTG CGT GTT CTT GGA CTC CGT CCC AAG GAA GAG TAGA
Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu

AGAAAAAAG CGCCGAATGA AGTATTGCC CCTTTTCTC CAAGAATGCC AAAAGGAGAT
CAAGTGGACA TTCTCTATGA AGA

9400306
S016
LPPNICH1H1YFOLBEMILBVDVQDQFCVLEYKVYPTFRANIASNHYRWUENHGRAS
LPPNICH1H1YFKNAPHLAEVDEBFQWHTALVHOLTFEGLUANYSMUKKHSINDET-----KAI EO 365

FIG. 8



Figure 9

Figure 10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07421

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N15/82 C12N5/10 C12P7/64 C11B1/00
A61K31/20 A23L1/30 A23K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12P C11B A61K A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO 93 06712 A (RHONE POULENC AGROCHIMIE) 15 April 1993 cited in the application see the whole document --- | 20-22 |
| X | WO 94 18337 A (MONSANTO CO ;UNIV MICHIGAN (US); GIBSON SUSAN IRMA (US); KISHORE G) 18 August 1994 * see the whole document, esp. claims 8-10 * | 20-47 |
| X | WO 96 21022 A (RHONE POULENC AGROCHIMIE) 11 July 1996 cited in the application * see the whole document, esp. p. 2 1.3-21 * --- -/-- | 20-47 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

21 August 1998

Date of mailing of the international search report

03/09/1998

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Authorized officer

Kania, T

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/07421

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/07421

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 23, 42, 43
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 98 /07421

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (group of) inventions in this international application, as follows:

1. Claims 1-47, 49,50

Nucleic acid constructs comprising delta-5, delta-6, or delta-12 desaturases according to SEQ ID NO: 1,3,5, derived from the fungus *Mortierella alpina*.

Recombinant plant cells comprising said constructs.

Methods for obtaining altered long chain polyunsaturated fatty acid biosynthesis using plants comprising delta-5, delta-6, or delta-12 desaturases, or combinations thereof, derived from fungi or algae.

Plant oils derived from said plants and their use for therapeutical, nutritional, and cosmetical purposes, as well as products derived therefrom.

2. Claim : 48

An isolated sequence comprising the nucleotide sequence selected from the group of SEQ ID NO: 38-44, wherein said nucleotide is expressed in a plant cells.

3. Claim : 51

An isolated nucleotide sequence selected from the group consisting of SEQ ID NO: 49-50, wherein said sequence is expressed in a plant cell.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/07421

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
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Information on patent family members

International Application No

PCT/US 98/07421

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶:C12N 15/53, 15/82, 5/10, C12P 7/64,
C11B 1/00, A61K 31/20, A23L 1/30,
A23K 1/00

A1

(11) International Publication Number:

WO 98/46764

(43) International Publication Date:

22 October 1998 (22.10.98)

(21) International Application Number: PCT/US98/07421

(22) International Filing Date: 10 April 1998 (10.04.98)

(30) Priority Data:

| | | |
|------------|----------------------------|----|
| 08/833,610 | 11 April 1997 (11.04.97) | US |
| 08/834,033 | 11 April 1997 (11.04.97) | US |
| 08/834,655 | 11 April 1997 (11.04.97) | US |
| 08/956,985 | 24 October 1997 (24.10.97) | US |

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

| | |
|----------|----------------------------|
| US | 08/834,655 (CIP) |
| Filed on | 11 April 1997 (11.04.97) |
| US | 08/833,610 (CIP) |
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| US | 08/834,033 (CIP) |
| Filed on | 11 April 1997 (11.04.97) |
| US | 08/956,985 (CIP) |
| Filed on | 24 October 1997 (24.10.97) |

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(75) Inventors/Applicants (for US only): KNUTZON, Deborah [US/US]; 6110 Rockhurst Way, Granite Bay, CA 95746 (US). MUKERJI, Pradip [US/US]; 1069 Arcaro Drive, Gahanna, OH 43230 (US). HUANG, Yung-Sheng [CA/US]; 2462 Danvers Court, Upper Arlington, OH 43220 (US). THURMOND, Jennifer [US/US]; 3702 Adirondack, Columbus, OH 43231 (US). CHAUDHARY, Sunita [IN/US]; 3419 Woodbine Place, Pearland, TX 77584 (US). LEONARD, Amanda, Eun-Yeong [US/US]; 581 Shadewood Court, Gahanna, OH 43230 (US).

(74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111-4262 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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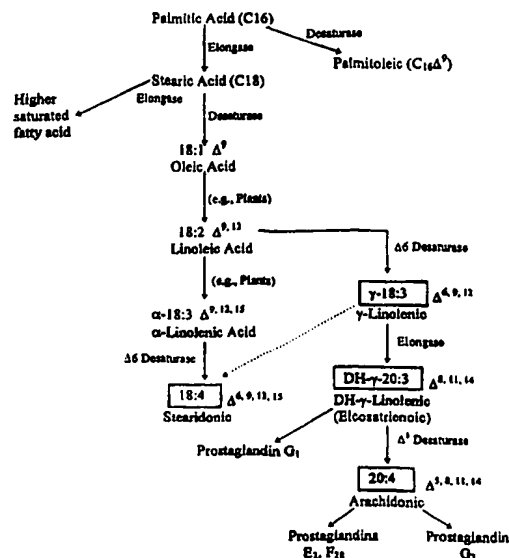
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

(57) Abstract

The present invention relates to compositions and methods for preparing polyunsaturated long chain fatty acids in plants, plant parts and plant cells, such as leaves, roots, fruits and seeds. Nucleic acid sequences and constructs encoding fatty acid desaturases, including $\Delta 5$ -desaturases, $\Delta 6$ -desaturases and $\Delta 12$ -desaturases, are used to generate transgenic plants, plant parts and cells which contain and express one or more transgenes encoding one or more desaturases. Expression of the desaturases with different substrate specificities in the plant system permit the large scale production of polyunsaturated long chain fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, α -linolenic acid, gamma-linolenic acid, arachidonic acid and the like for modification of the fatty acid profile of plants, plant parts and tissues. Manipulation of the fatty acid profiles allows for the production of commercial quantities of novel plant oils and products.



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| EE | Estonia | | | | | | |



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶:C12N 15/53, 15/82, 5/10, C12P 7/64,
C11B 1/00, A61K 31/20, A23L 1/30,
A23K 1/00

A1

(11) International Publication Number:

WO 98/46764

(43) International Publication Date:

22 October 1998 (22.10.98)

(21) International Application Number: PCT/US98/07421

(22) International Filing Date: 10 April 1998 (10.04.98)

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ABBOTT LABORATORIES [US/US]; 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

(72) Inventors; and

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

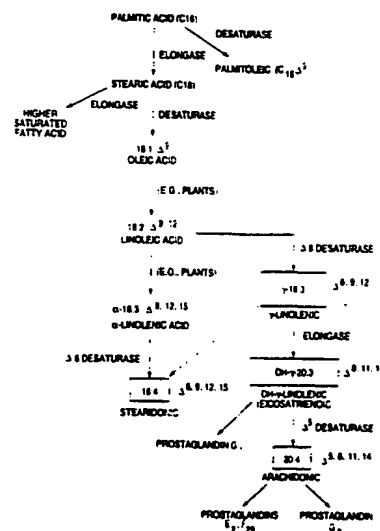
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

(57) Abstract

The present invention relates to compositions and methods for preparing polyunsaturated long chain fatty acids in plants, plant parts and plant cells, such as leaves, roots, fruits and seeds. Nucleic acid sequences and constructs encoding fatty acid desaturases, including $\Delta 5$ -desaturases, $\Delta 6$ -desaturases and $\Delta 12$ -desaturases, are used to generate transgenic plants, plant parts and cells which contain and express one or more transgenes encoding one or more desaturases. Expression of the desaturases with different substrate specificities in the plant system permit the large scale production of polyunsaturated long chain fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, α -linolenic acid, gamma-linolenic acid, arachidonic acid and the like for modification of the fatty acid profile of plants, plant parts and tissues. Manipulation of the fatty acid profiles allows for the production of commercial quantities of novel plant oils and products.



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METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of USSN 08/834,655, filed
5 April 11, 1997, and a continuation in part of USSN 08/833,610, filed April 11,
1997, USSN 08/834,033 filed April 11, 1997 and USSN 08/956,985 filed
October 24, 1997 which disclosures are incorporated herein by reference.

INTRODUCTION

Field of the Invention

10 This invention relates to modulating levels of enzymes and/or enzyme
components capable of altering the production of long chain polyunsaturated
fatty acids (PUFAS) in a host plant. The invention is exemplified by the
production of PUFAS in plants.

Background

15 Two main families of polyunsaturated fatty acids (PUFAs) are the $\omega 3$
fatty acids, exemplified by arachidonic acid, and the $\omega 6$ fatty acids, exemplified
by eicosapentaenoic acid. PUFAs are important components of the plasma
membrane of the cell, where they may be found in such forms as phospholipids.
PUFAs also serve as precursors to other molecules of importance in human
20 beings and animals, including the prostacyclins, leukotrienes and
prostaglandins. PUFAs are necessary for proper development, particularly in
the developing infant brain, and for tissue formation and repair.

Four major long chain PUFAs of importance include docosahexaenoic
acid (DHA) and eicosapentaenoic acid (EPA), which are primarily found in
25 different types of fish oil, gamma-linolenic acid (GLA), which is found in the
seeds of a number of plants, including evening primrose (*Oenothera biennis*),
borage (*Borago officinalis*) and black currants (*Ribes nigrum*), and stearidonic
acid (SDA), which is found in marine oils and plant seeds. Both GLA and
another important long chain PUFA, arachidonic acid (ARA), are found in

filamentous fungi. ARA can be purified from animal tissues including liver and adrenal gland.

For DHA, a number of sources exist for commercial production including a variety of marine organisms, oils obtained from cold water marine fish, and egg yolk fractions. For ARA, microorganisms including the genera *Mortierella*, *Entomophthora*, *Phytium* and *Porphyridium* can be used for commercial production. Commercial sources of SDA include the genera *Trichodesma* and *Echium*. Commercial sources of GLA include evening primrose, black currants and borage. However, there are several disadvantages associated with commercial production of PUFAs from natural sources. Natural sources of PUFAs, such as animals and plants, tend to have highly heterogeneous oil compositions. The oils obtained from these sources therefore can require extensive purification to separate out one or more desired PUFAs or to produce an oil which is enriched in one or more PUFA. Natural sources also are subject to uncontrollable fluctuations in availability. Fish stocks may undergo natural variation or may be depleted by overfishing. Fish oils have unpleasant tastes and odors, which may be impossible to economically separate from the desired product, and can render such products unacceptable as food supplements. Animal oils, and particularly fish oils, can accumulate environmental pollutants. Weather and disease can cause fluctuation in yields from both fish and plant sources. Cropland available for production of alternate oil-producing crops is subject to competition from the steady expansion of human populations and the associated increased need for food production on the remaining arable land. Crops which do produce PUFAs, such as borage, have not been adapted to commercial growth and may not perform well in monoculture. Growth of such crops is thus not economically competitive where more profitable and better established crops can be grown. Large scale fermentation of organisms such as *Mortierella* is also expensive. Natural animal tissues contain low amounts of ARA and are difficult to process. Microorganisms such as *Porphyridium* and *Mortierella* are difficult to cultivate on a commercial scale.

Dietary supplements and pharmaceutical formulations containing PUFAs can retain the disadvantages of the PUFA source. Supplements such as fish oil capsules can contain low levels of the particular desired component and thus require large dosages. High dosages result in ingestion of high levels of
5 undesired components, including contaminants. Care must be taken in providing fatty acid supplements, as overaddition may result in suppression of endogenous biosynthetic pathways and lead to competition with other necessary fatty acids in various lipid fractions *in vivo*, leading to undesirable results. For example, Eskimos having a diet high in ω 3 fatty acids have an increased
10 tendency to bleed (U.S. Pat. No. 4,874,603). Unpleasant tastes and odors of the supplements can make such regimens undesirable, and may inhibit compliance by the patient.

A number of enzymes are involved in PUFA biosynthesis. Linoleic acid (LA, 18:2 Δ 9, 12) is produced from oleic acid (18:1 Δ 9) by a Δ 12-desaturase.
15 GLA (18:3 Δ 6, 9, 12) is produced from linoleic acid (LA, 18:2 Δ 9, 12) by a Δ 6-desaturase. ARA (20:4 Δ 5, 8, 11, 14) production from DGLA (20:3 Δ 8, 11, 14) is catalyzed by a Δ 5-desaturase. However, animals cannot desaturate beyond the Δ 9 position and therefore cannot convert oleic acid (18:1 Δ 9) into linoleic acid (18:2 Δ 9, 12). Likewise, α -linolenic acid (ALA, 18:3 Δ 9, 12, 15) cannot
20 be synthesized by mammals. Other eukaryotes, including fungi and plants, have enzymes which desaturate at positions Δ 21 and Δ 15. The major poly-unsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid (18:2 Δ 9, 12) or α -linolenic acid (18:3 Δ 9, 12, 15).

25 Poly-unsaturated fatty acids are considered to be useful for nutritional, pharmaceutical, industrial, and other purposes. An expansive supply of poly-unsaturated fatty acids from natural sources and from chemical synthesis are not sufficient for commercial needs. Therefore it is of interest to obtain genetic material involved in PUFA biosynthesis from species that naturally produce
30 these fatty acids and to express the isolated material alone or in combination in

a heterologous system which can be manipulated to allow production of commercial quantities of PUFAS.

5 The present invention is further directed to formulas, dietary supplements or dietary supplements in the form of a liquid or a solid containing the long chain fatty acids of the invention. These formulas and supplements may be administered to a human or an animal.

10 The formulas and supplements of the invention may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

15 The formulas of the present invention may further include at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

20 The present invention is further directed to a method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient a dietary substitute of the invention in an amount sufficient to effect treatment of the patient.

The present invention is further directed to cosmetic and pharmaceutical compositions of the material of the invention.

25 The present invention is further directed to transgenic oils in pharmaceutically acceptable carriers. The present invention is further directed to nutritional supplements, cosmetic agents and infant formulae containing transgenic oils.

The present invention is further directed to a method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of: growing a microbe having cells which contain a transgene which encodes a

transgene expression product which desaturates a fatty acid molecule at carbon 5,5 or 12 from the carboxyl end of said fatty acid molecule, wherein the transgene is operably associated with an expression control sequence, under conditions whereby the transgene is expressed, whereby long chain
5 polyunsaturated fatty acid biosynthesis in the cells is altered.

The present invention is further directed toward pharmaceutical compositions comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

10 Relevant Literature

Production of gamma-linolenic acid by a $\Delta 6$ -desaturase is described in USPN 5,552,306 and USPN 5,614,393. Production of 8, 11-eicosadienoic acid using *Mortierella alpina* is disclosed in USPN 5,376,541. Production of docosahexaenoic acid by dinoflagellates is described in USPN 5,407,957.
15 Cloning of a $\Delta 6$ -desaturase from borage is described in PCT publication WO 96/21022. Cloning of $\Delta 9$ -desaturases is described in the published patent applications PCT WO 91/13972, EP 0 550 162 A1, EP 0 561 569 A2, EP 0 644 263 A2, and EP 0 736 598 A1, and in USPN 5,057,419. Cloning of $\Delta 12$ -desaturases from various organisms is described in PCT publication WO
20 94/11516 and USPN 5,443,974. Cloning of $\Delta 15$ -desaturases from various organisms is described in PCT publication WO 93/11245. A $\Delta 6$ palmitoyl-acyl carrier protein desaturase from *Thumbergia alata* and its expression in *E. coli* is described in USPN 5,614,400. Expression of a soybean stearyl-ACP desaturase in transgenic soybean embryos using a 35S promoter is disclosed in USPN
25 5,443,974.

SUMMARY OF THE INVENTION

Novel compositions and methods are provided for preparation of poly-unsaturated long chain fatty acids and desaturases in plants and plant cells. The methods involve growing a host plant cell of interest transformed with an
30 expression cassette functional in a host plant cell, the expression cassette

comprising a transcriptional and translational initiation regulatory region, joined in reading frame 5' to a DNA sequence encoding a desaturase polypeptide capable of modulating the production of PUFAs. Expression of the desaturase polypeptide provides for an alteration in the PUFA profile of host plant cells as a result of altered concentrations of enzymes involved in PUFA biosynthesis. Of particular interest is the selective control of PUFA production in plant tissues and/or plant parts such as leaves, roots, fruits and seeds. The invention finds use for example in the large scale production of DHA, EPA, ARA, and GLA and for modification of the fatty acid profile of edible plant tissues and/or plant parts.

The present invention further includes a purified nucleotide sequence or polypeptide sequence that is substantially related or homologous to the nucleotide and peptide sequences presented in SEQ ID NO:1 - SEQ ID NO:52. The present invention is further directed to methods of using the sequences presented in SEQ ID NO:1 to SEQ ID NO:40 as probes to identify related sequences, as components of expression systems and as components of systems useful for producing transgenic oil.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows possible pathways for the synthesis of arachidonic acid (20:4 Δ 5, 8, 11, 14) and stearidonic acid (18:4 Δ 6, 9, 12, 15) from palmitic acid (C_{16}) from a variety of organisms, including algae, *Mortierella* and humans. These PUFAs can serve as precursors to other molecules important for humans and other animals, including prostacyclins, leukotrienes, and prostaglandins, some of which are shown.

Figure 2 shows possible pathways for production of PUFAs in addition to ARA, including EPA and DHA, again compiled from a variety of organisms.

Figure 3A-E shows the DNA sequence (SEQ ID NO:1) of the *Mortierella alpina* Δ 6 desaturase and the deduced amino acid sequence (SEQ ID NO:2).

Figure 4 shows an alignment of the *Mortierella alpina* $\Delta 6$ desaturase amino acid sequence with other $\Delta 6$ desaturases and related sequences (SEQ ID NOS:7, 8, 9, 10, 11, 12 and 13).

5 Figure 5A-D shows the DNA sequence of the *Mortierella alpina* $\Delta 12$ desaturase (SEQ ID NO:3) and the deduced amino acid sequence (SEQ ID NO:4)

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:14) of the PCR fragment (see Example 1).

10 Figure 7A-D shows the DNA sequence of the *Mortierella alpina* $\Delta 5$ desaturase (SEQ ID NO:5).

Figure 8 shows alignments of the protein sequence of the $\Delta 5$ desaturase (SEQ ID NO:6) with $\Delta 6$ desaturases and related sequences (SEQ ID NOS:15, 16, 17, 18).

15 Figure 9 shows alignments of the protein sequence of the Ma 29 and contig 253538a.

Figure 10 shows alignments of the protein sequence of Ma 524 and contig 253538a.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

20 SEQ ID NO:1 shows the DNA sequence of the *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:2 shows the amino acid sequence of the *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:3 shows the DNA sequence of the *Mortierella alpina* $\Delta 12$ desaturase.

25 SEQ ID NO:4 shows the amino acid sequence of the *Mortierella alpina* $\Delta 12$ desaturase.

SEQ ID NO:5 shows the DNA sequence of the *Mortierella alpina* $\Delta 5$ desaturase.

SEQ ID NO:6 shows the amino acid sequence *Mortierella alpina* $\Delta 5$ desaturase.

5 SEQ ID NO:7 - SEQ ID NO:13 show amino acid sequences that relate to *Mortierella alpina* $\Delta 6$ desaturase.

SEQ ID NO:14 shows an amino acid sequence of a PCR fragment of Example 1.

10 SEQ ID NO:15 - SEQ ID NO:18 show amino acid sequences that relate to *Mortierella alpina* $\Delta 5$ and $\Delta 6$ desaturases.

SEQ ID NO:19 - SEQ ID NO:30 show PCR primer sequences.

SEQ ID NO:31 - SEQ ID NO:37 show human nucleotide sequences.

SEQ ID NO:38 - SEQ ID NO:44 show human peptide sequences.

15 SEQ ID NO:45 - SEQ ID NO:46 show the nucleotide and amino acid sequence of a *Dictyostelium discoideum* desaturase.

SEQ ID NO:47 - SEQ ID NO:50 show the nucleotide and deduced amino acid sequence of a *Schizochytrium* cDNA clone.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 In order to ensure a complete understanding of the invention, the following definitions are provided:

$\Delta 5$ -Desaturase: $\Delta 5$ desaturase is an enzyme which introduces a double bond between carbons 5 and 6 from the carboxyl end of a fatty acid molecule.

$\Delta 6$ -Desaturase: $\Delta 6$ -desaturase is an enzyme which introduces a double bond between carbons 6 and 7 from the carboxyl end of a fatty acid molecule.

25 **$\Delta 9$ -Desaturase:** $\Delta 9$ -desaturase is an enzyme which introduces a double bond between carbons 9 and 10 from the carboxyl end of a fatty acid molecule.

$\Delta 12$ -Desaturase: $\Delta 12$ -desaturase is an enzyme which introduces a double bond between carbons 12 and 13 from the carboxyl end of a fatty acid molecule.

Fatty Acids: Fatty acids are a class of compounds containing a long hydrocarbon chain and a terminal carboxylate group. Fatty acids include the following:

| Fatty Acid | | |
|----------------------|--|---|
| 12:0 | lauric acid | |
| 16:0 | palmitic acid | |
| 16:1 | palmitoleic acid | |
| 18:0 | stearic acid | |
| 18:1 | oleic acid | $\Delta 9-18:1$ |
| 18:2 $\Delta 5,9$ | taxoleic acid | $\Delta 5,9-18:2$ |
| 18:2 $\Delta 6,9$ | 6,9-octadecadienoic acid | $\Delta 6,9-18:2$ |
| 18:2 | linoleic acid | $\Delta 9,12-18:2$ (LA) |
| 18:3 $\Delta 6,9,12$ | gamma-linolenic acid | $\Delta 6,9,12-18:3$ (GLA) |
| 18:3 $\Delta 5,9,12$ | pinolenic acid | $\Delta 5,9,12-18:3$ |
| 18:3 | alpha-linolenic acid | $\Delta 9,12,15-18:3$ (ALA) |
| 18:4 | stearidonic acid | $\Delta 6,9,12,15-18:4$ (SDA) |
| 20:0 | Arachidic acid | |
| 20:1 | Eicosenic Acid | |
| 22:0 | behehic acid | |
| 22:1 | erucic acid | |
| 22:2 | Docasadienoic acid | |
| 20:4 $\omega 6$ | arachidonic acid | $\Delta 5,8,11,14-20:4$ (ARA) |
| 20:3 $\omega 6$ | $\omega 6$ -eicosatrienoic dihomogamma linolenic | $\Delta 8,11,14-20:3$ (DGLA) |
| 20:5 $\omega 3$ | Eicosapentanoic (Timnodonic acid) | $\Delta 5,8,11,14,17-20:5$ (EPA) |
| 20:3 $\omega 3$ | $\omega 3$ -eicosatrienoic | $\Delta 11,16,17-20:3$ |
| 20:4 $\omega 3$ | $\omega 3$ -eicosatetraenoic | $\Delta 8,11,14,17-20:4$ |
| 22:5 $\omega 3$ | Docosapentaenoic | $\Delta 7,10,13,16,19-22:5$ ($\omega 3$ DPA) |
| 22:6 $\omega 3$ | Docosahexaenoic (cervonic acid) | $\Delta 4,7,10,13,16,19-22:6$ (DHA) |
| 24:0 | Lignoceric acid | |

Taking into account these definitions, the present invention is directed to novel DNA sequences, DNA constructs, methods and compositions are provided which permit modification of the poly-unsaturated long chain fatty acid content of plant cells. Plant cells are transformed with an expression cassette

5 comprising a DNA encoding a polypeptide capable of increasing the amount of one or more PUFA in a plant cell. Desirably, integration constructs may be prepared which provide for integration of the expression cassette into the genome of a host cell. Host cells are manipulated to express a sense or antisense DNA encoding a polypeptide(s) that has desaturase activity. By

10 "desaturase" is intended a polypeptide which can desaturate one or more fatty acids to produce a mono- or poly-unsaturated fatty acid or precursor thereof of interest. By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification, for example, glycosylation or phosphorylation. The substrate(s) for the expressed enzyme may be produced

15 by the host cell or may be exogenously supplied.

To achieve expression in a host cell, the transformed DNA is operably associated with transcriptional and translational initiation and termination regulatory regions that are functional in the host cell. Constructs comprising the gene to be expressed can provide for integration into the genome of the host cell

20 or can autonomously replicate in the host cell. For production of linoleic acid (LA), the expression cassettes generally used include a cassette which provides for $\Delta 12$ desaturase activity, particularly in a host cell which produces or can take up oleic acid. For production of ALA, the expression cassettes generally used include a cassette which provides for $\Delta 15$ or $\omega 3$ desaturase activity,

25 particularly in a host cell which produces or can take up LA. For production of GLA or SDA, the expression cassettes generally used include a cassette which provides for $\Delta 6$ desaturase activity, particularly in a host cell which produces or can take up LA or ALA, respectively. Production of $\omega 6$ -type unsaturated fatty acids, such as LA or GLA, is favored in a plant capable of producing ALA by

30 inhibiting the activity of a $\Delta 15$ or $\omega 3$ type desaturase; this is accomplished by providing an expression cassette for an antisense $\Delta 15$ or $\omega 3$ transcript, or by

disrupting a $\Delta 15$ or $\omega 3$ desaturase gene. Similarly, production of LA or ALA is favored in a plant having $\Delta 6$ desaturase activity by providing an expression cassette for an antisense $\Delta 6$ transcript, or by disrupting a $\Delta 6$ desaturase gene. Production of oleic acid likewise is favored in a plant having $\Delta 12$ desaturase activity by providing an expression cassette for an antisense $\Delta 12$ transcript, or by disrupting a $\Delta 12$ desaturase gene. For production of ARA, the expression cassette generally used provides for $\Delta 5$ desaturase activity, particularly in a host cell which produces or can take up DGLA. Production of $\omega 6$ -type unsaturated fatty acids, such as ARA, is favored in a plant capable of producing ALA by inhibiting the activity of a $\Delta 15$ or $\omega 3$ type desaturase; this is accomplished by providing an expression cassette for an antisense $\Delta 15$ or $\omega 3$ transcript, or by disrupting a $\Delta 15$ or $\omega 3$ desaturase gene.

TRANSGENIC PLANT PRODUCTION OF FATTY ACIDS

Transgenic plant production of PUFAs offers several advantages over purification from natural sources such as fish or plants. Production of fatty acids from recombinant plants provides the ability to alter the naturally occurring plant fatty acid profile by providing new synthetic pathways in the host or by suppressing undesired pathways, thereby increasing levels of desired PUFAs, or conjugated forms thereof, and decreasing levels of undesired PUFAs. Production of fatty acids in transgenic plants also offers the advantage that expression of desaturase genes in particular tissues and/or plant parts means that greatly increased levels of desired PUFAs in those tissues and/or parts can be achieved, making recovery from those tissues more economical. For example, the desired PUFAs can be expressed in seed; methods of isolating seed oils are well established. In addition to providing a source for purification of desired PUFAs, seed oil components can be manipulated through expression of desaturase genes, either alone or in combination with other genes such as elongases, to provide seed oils having a particular PUFA profile in concentrated form. The concentrated seed oils then can be added to animal milks and/or synthetic or semi-synthetic milks to serve as infant formulas where human

nursing is impossible or undesired, or in cases of malnourishment or disease in both adults and infants.

For production of PUFAs, depending upon the host cell, the availability of substrate, and the desired end product(s), several polypeptides, particularly
5 desaturases, are of interest including those polypeptides which catalyze the conversion of stearic acid to oleic acid, LA to GLA, of ALA to SDA, of oleic acid to LA, or of LA to ALA, which includes enzymes which desaturate at the $\Delta 6$, $\Delta 9$, $\Delta 12$, $\Delta 15$ or $\omega 3$ positions. Considerations for choosing a specific polypeptide having desaturase activity include the pH optimum of the
10 polypeptide, whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired polyunsaturated fatty acid, and/or co-factors required by the polypeptide. The expressed polypeptide preferably has parameters compatible with the biochemical environment of its location in the host cell. For example, the
15 polypeptide may have to compete for substrate with other enzymes in the host cell. Analyses of the K_m and specific activity of the polypeptide in question therefore are considered in determining the suitability of a given polypeptide for modifying PUFA production in a given host cell. The polypeptide used in a particular situation therefore is one which can function under the conditions
20 present in the intended host cell but otherwise can be any polypeptide having desaturase activity which has the desired characteristic of being capable of modifying the relative production of a desired PUFA. A scheme for the synthesis of arachidonic acid (20:4 $\Delta 5$, 8, 11, 14) from palmitic acid (C_{16}) is shown in Figure 1. A key enzyme in this pathway is a $\Delta 5$ -desaturase which
25 converts DH- γ -linolenic acid (DGLA, eicosatrienoic acid) to ARA. Conversion of α -linolenic acid (ALA) to stearidonic acid by a $\Delta 6$ -desaturase is also shown. Production of PUFAs in addition to ARA, including EPA and DHA is shown in Figure 2. A key enzyme in the synthesis of arachidonic acid (20:4 $\Delta 5$, 8, 11, 14) from stearic acid (C_{18}) is a $\Delta 6$ -desaturase which converts the linoleic acid
30 into γ -linolenic acid. Conversion of α -linolenic acid (ALA) to stearidonic acid by a $\Delta 6$ -desaturase also is shown. For production of ARA, the DNA sequence

used encodes a polypeptide having $\Delta 5$ desaturase activity. In particular instances, this can be coupled with an expression cassette which provides for production of a polypeptide having $\Delta 6$ desaturase activity and, optionally, a transcription cassette providing for production of antisense sequences to a Δ 15 transcription product. The choice of combination of cassettes used depends in part on the PUFA profile of the host cell. Where the host cell $\Delta 5$ -desaturase activity is limiting, overexpression of $\Delta 5$ desaturase alone generally will be sufficient to provide for enhanced ARA production.

10 SOURCES OF POLYPEPTIDES HAVING DESATURASE ACTIVITY

As sources of polypeptides having desaturase activity and oligonucleotides encoding such polypeptides are organisms which produce a desired poly-unsaturated fatty acid. As an example, microorganisms having an ability to produce ARA can be used as a source of $\Delta 5$ -desaturase genes; 15 microorganisms which GLA or SDA can be used as a source of $\Delta 6$ -desaturase and/or $\Delta 12$ -desaturase genes. Such microorganisms include, for example, those belonging to the genera *Mortierella*, *Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor*, *Fusarium*, *Aspergillus*, *Rhodotorula*, and *Entomophthora*. Within the genus *Porphyridium*, of particular interest is *Porphyridium cruentum*. Within the genus *Mortierella*, of 20 particular interest are *Mortierella elongata*, *Mortierella exigua*, *Mortierella hygrophila*, *Mortierella ramanniana*, var. *angulispora*, and *Mortierella alpina*. Within the genus *Mucor*, of particular interest are *Mucor circinelloides* and *Mucor javanicus*.

25 DNAs encoding desired desaturases can be identified in a variety of ways. As an example, a source of the desired desaturase, for example genomic or cDNA libraries from *Mortierella*, is screened with detectable enzymatically- or chemically-synthesized probes, which can be made from DNA, RNA, or non-naturally occurring nucleotides, or mixtures thereof. Probes may be 30 enzymatically synthesized from DNAs of known desaturases for normal or

reduced-stringency hybridization methods. Oligonucleotide probes also can be used to screen sources and can be based on sequences of known desaturases, including sequences conserved among known desaturases, or on peptide sequences obtained from the desired purified protein. Oligonucleotide probes
5 based on amino acid sequences can be degenerate to encompass the degeneracy of the genetic code, or can be biased in favor of the preferred codons of the source organism. Oligonucleotides also can be used as primers for PCR from reverse transcribed mRNA from a known or suspected source; the PCR product can be the full length cDNA or can be used to generate a probe to obtain the
10 desired full length cDNA. Alternatively, a desired protein can be entirely sequenced and total synthesis of a DNA encoding that polypeptide performed.

Once the desired genomic or cDNA has been isolated, it can be sequenced by known methods. It is recognized in the art that such methods are subject to errors, such that multiple sequencing of the same region is routine and
15 is still expected to lead to measurable rates of mistakes in the resulting deduced sequence, particularly in regions having repeated domains, extensive secondary structure, or unusual base compositions, such as regions with high GC base content. When discrepancies arise, resequencing can be done and can employ special methods. Special methods can include altering sequencing conditions
20 by using: different temperatures; different enzymes; proteins which alter the ability of oligonucleotides to form higher order structures; altered nucleotides such as ITP or methylated dGTP; different gel compositions, for example adding formamide; different primers or primers located at different distances from the problem region; or different templates such as single stranded DNAs.
25 Sequencing of mRNA can also be employed.

For the most part, some or all of the coding sequence for the polypeptide having desaturase activity is from a natural source. In some situations, however, it is desirable to modify all or a portion of the codons, for example, to enhance expression, by employing host preferred codons. Host preferred
30 codons can be determined from the codons of highest frequency in the proteins expressed in the largest amount in a particular host species of interest. Thus, the

coding sequence for a polypeptide having desaturase activity can be synthesized in whole or in part. All or portions of the DNA also can be synthesized to remove any destabilizing sequences or regions of secondary structure which would be present in the transcribed mRNA. All or portions of the DNA also can be synthesized to alter the base composition to one more preferable in the desired host cell. Methods for synthesizing sequences and bringing sequences together are well established in the literature. *In vitro* mutagenesis and selection, site-directed mutagenesis, or other means can be employed to obtain mutations of naturally occurring desaturase genes to produce a polypeptide having desaturase activity *in vivo* with more desirable physical and kinetic parameters for function in the host cell, such as a longer half-life or a higher rate of production of a desired polyunsaturated fatty acid.

Desirable cDNAs have less than 60% A+T composition, preferably less than 50% A+T composition. On a localized scale of a sliding window of 20 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 75% A+T composition; with a window of 60 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 60%, more preferably no localized regions with greater than 55% A+T composition.

Mortierella alpina Desaturases

Of particular interest are the *Mortierella alpina* $\Delta 5$ -desaturase, $\Delta 6$ -desaturase and $\Delta 12$ -desaturase. The $\Delta 5$ -desaturase has 446 amino acids; the amino acid sequence is shown in Figure 7. The gene encoding the *Mortierella alpina* $\Delta 5$ -desaturase can be expressed in transgenic microorganisms to effect greater synthesis of ARA from DGLA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina* $\Delta 5$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina* $\Delta 5$ -desaturase polypeptide, also can be used. The *Mortierella alpina* $\Delta 6$ -desaturase, has 457 amino acids and a predicted molecular weight of 51.8 kD; the amino acid sequence is shown in Figure 3.

The gene encoding the *Mortierella alpina* $\Delta 6$ -desaturase can be expressed in transgenic plants or animals to effect greater synthesis of GLA from linoleic acid or of stearidonic acid (SDA) from ALA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina* $\Delta 6$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina* $\Delta 6$ -desaturase polypeptide, also can be used.

The *Mortierella alpina* $\Delta 12$ -desaturase has the amino acid sequence shown in Figure 5. The gene encoding the *Mortierella alpina* $\Delta 12$ -desaturase can be expressed in transgenic plants to effect greater synthesis of LA from oleic acid. Other DNAs which are substantially identical to the *Mortierella alpina* $\Delta 12$ -desaturase DNA, or which encode polypeptides which are substantially identical to the *Mortierella alpina* $\Delta 12$ -desaturase polypeptide, also can be used.

By substantially identical in sequence is intended an amino acid sequence or nucleic acid sequence exhibiting in order of increasing preference at least 60%, 80%, 90% or 95% homology to the *Mortierella alpina* $\Delta 5$ -desaturase amino acid sequence or nucleic acid sequence encoding the amino acid sequence. For polypeptides, the length of comparison sequences generally is at least 16 amino acids, preferably at least 20 amino acids, or most preferably 35 amino acids. For nucleic acids, the length of comparison sequences generally is at least 50 nucleotides, preferably at least 60 nucleotides, and more preferably at least 75 nucleotides, and most preferably, 110 nucleotides. Homology typically is measured using sequence analysis software, for example, the Sequence Analysis software package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wisconsin 53705, MEGAlign (DNASStar, Inc., 1228 S. Park St., Madison, Wisconsin 53715), and MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, California 95008). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine and alanine;

valine, isoleucine and leucine; aspartic acid, glutamic acid, asparagine, and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Substitutions may also be made on the basis of conserved hydrophobicity or hydrophilicity (Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132, 1982), or on the basis of the ability to assume similar polypeptide secondary structure (Chou and Fasman, *Adv. Enzymol.* 47: 45-148, 1978).

Other Desaturases

Encompassed by the present invention are related desaturases from the same or other organisms. Such related desaturases include variants of the disclosed $\Delta 5$ -, $\Delta 6$ - and $\Delta 12$ -desaturases that occur naturally within the same or different species of *Mortierella*, as well as homologues of the disclosed $\Delta 5$ -desaturase from other species and evolutionarily related protein having desaturase activity. Also included are desaturases which, although not substantially identical to the *Mortierella alpina* $\Delta 5$ -desaturase, desaturate a fatty acid molecule at carbon 5, 6 or 12, respectively, from the carboxyl end of a fatty acid molecule. Related desaturases can be identified by their ability to function substantially the same as the disclosed desaturases; that is, are still able to effectively convert DGLA to ARA, LA to GLA, ALA to SDA or oleic acid to LA. Related desaturases also can be identified by screening sequence databases for sequences homologous to the disclosed desaturase, by hybridization of a probe based on the disclosed desaturase to a library constructed from the source organism, or by RT-PCR using mRNA from the source organism and primers based on the disclosed desaturase. Such desaturases includes those from humans, *Dictyostelium discoideum* and *Phaeodactylum tricornutum*.

The regions of a desaturase polypeptide important for desaturase activity can be determined through routine mutagenesis, expression of the resulting mutant polypeptides and determination of their activities. Mutants may include deletions, insertions and point mutations, or combinations thereof. A typical functional analysis begins with deletion mutagenesis to determine the N- and C-terminal limits of the protein necessary for function, and then internal deletions,

insertions or point mutants are made to further determine regions necessary for function. Other techniques such as cassette mutagenesis or total synthesis also can be used. Deletion mutagenesis is accomplished, for example, by using exonucleases to sequentially remove the 5' or 3' coding regions. Kits are
5 available for such techniques. After deletion, the coding region is completed by ligating oligonucleotides containing start or stop codons to the deleted coding region after 5' or 3' deletion, respectively. Alternatively, oligonucleotides encoding start or stop codons are inserted into the coding region by a variety of methods including site-directed mutagenesis, mutagenic PCR or by ligation
10 onto DNA digested at existing restriction sites. Internal deletions can similarly be made through a variety of methods including the use of existing restriction sites in the DNA, by use of mutagenic primers via site directed mutagenesis or mutagenic PCR. Insertions are made through methods such as linker-scanning mutagenesis, site-directed mutagenesis or mutagenic PCR. Point mutations are
15 made through techniques such as site-directed mutagenesis or mutagenic PCR.

Chemical mutagenesis can also be used for identifying regions of a desaturase polypeptide important for activity. A mutated construct is expressed, and the ability of the resulting altered protein to function as a desaturase is assayed. Such structure-function analysis can determine which regions may be
20 deleted, which regions tolerate insertions, and which point mutations allow the mutant protein to function in substantially the same way as the native desaturase. All such mutant proteins and nucleotide sequences encoding them are within the scope of the present invention.

EXPRESSION OF DESATURASE GENES

25 Once the DNA encoding a desaturase polypeptide has been obtained, it is placed in a vector capable of replication in a host cell, or is propagated *in vitro* by means of techniques such as PCR or long PCR. Replicating vectors can include plasmids, phage, viruses, cosmids and the like. Desirable vectors include those useful for mutagenesis of the gene of interest or for expression of
30 the gene of interest in host cells. The technique of long PCR has made *in vitro* propagation of large constructs possible, so that modifications to the gene of

interest, such as mutagenesis or addition of expression signals, and propagation of the resulting constructs can occur entirely *in vitro* without the use of a replicating vector or a host cell.

For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is easily harvested, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location within the plant by using specific regulatory sequences, such as those of USPN 5,463,174, USPN 4,943,674, USPN 5,106,739, USPN 5,175,095, USPN 5,420,034, USPN 5,188,958, and USPN 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. In the present case, expression of desaturase genes, or antisense desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The $\Delta 5$ -desaturase polypeptide coding region is expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto *et al.*, PCT publication WO 95/24494). The termination region can be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected more as a matter of convenience rather than because of any particular property.

The choice of a host cell is influenced in part by the desired PUFA profile of the transgenic cell, and the native profile of the host cell. As an example, for production of linoleic acid from oleic acid, the DNA sequence used encodes a polypeptide having $\Delta 12$ desaturase activity, and for production of GLA from linoleic acid, the DNA sequence used encodes a polypeptide having $\Delta 6$ desaturase activity. Use of a host cell which expresses $\Delta 12$ desaturase activity and lacks or is depleted in $\Delta 15$ desaturase activity, can be used with an expression cassette which provides for overexpression of $\Delta 6$ desaturase alone generally is sufficient to provide for enhanced GLA production in the transgenic cell. Where the host cell expresses $\Delta 9$ desaturase activity, expression of both a $\Delta 12$ - and a $\Delta 6$ -desaturase can provide for enhanced GLA production. In particular instances where expression of $\Delta 6$ desaturase activity is coupled with expression of $\Delta 12$ desaturase activity, it is desirable that the host cell naturally have, or be mutated to have, low $\Delta 15$ desaturase activity. Alternatively, a host cell for $\Delta 6$ desaturase expression may have, or be mutated to have, high $\Delta 12$ desaturase activity.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, integration of constructs can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to

target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

When increased expression of the desaturase polypeptide in the source
5 plant is desired, several methods can be employed. Additional genes encoding the desaturase polypeptide can be introduced into the host organism. Expression from the native desaturase locus also can be increased through homologous recombination, for example by inserting a stronger promoter into the host genome to cause increased expression, by removing destabilizing
10 sequences from either the mRNA or the encoded protein by deleting that information from the host genome, or by adding stabilizing sequences to the mRNA (*see* USPN 4,910,141 and USPN 5,500,365.)

When it is desirable to express more than one different gene, appropriate regulatory regions and expression methods, introduced genes can be propagated
15 in the host cell through use of replicating vectors or by integration into the host genome. Where two or more genes are expressed from separate replicating vectors, it is desirable that each vector has a different means of replication. Each introduced construct, whether integrated or not, should have a different means of selection and should lack homology to the other constructs to maintain
20 stable expression and prevent reassortment of elements among constructs. Judicious choices of regulatory regions, selection means and method of propagation of the introduced construct can be experimentally determined so that all introduced genes are expressed at the necessary levels to provide for synthesis of the desired products.

25 Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. These techniques include transfection, infection, bolistic impact, electroporation, microinjection, scraping, or any other method which introduces the gene of interest into the host cell (*see* USPN 4,743,548, USPN 4,795,855, USPN 5,068,193, USPN 5,188,958, USPN 5,463,174, USPN
30 5,565,346 and USPN 5,565,347). For convenience, a host cell which has been manipulated by any method to take up a DNA sequence or construct will be

referred to as "transformed" or "recombinant" herein. The subject host will have at least have one copy of the expression construct and may have two or more, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy
5 numbers.

The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a separate marker construct may be introduced with the desired construct, as many transformation techniques introduce many DNA molecules into host cells. Typically,
10 transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene therefor may confer antibiotic resistance, or encode an essential growth factor or enzyme, and permit growth on selective media when
15 expressed in the transformed host cell. Desirably, resistance to kanamycin and the amino glycoside G418 are of interest (*see* USPN 5,034,322). Selection of a transformed host can also occur when the expressed marker protein can be detected, either directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by
20 its enzymatic activity; for example β galactosidase can convert the substrate X-gal to a colored product, and luciferase can convert luciferin to a light-emitting product. The marker protein can be detected by its light-producing or modifying characteristics; for example, the green fluorescent protein of *Aequorea victoria* fluoresces when illuminated with blue light. Antibodies can
25 be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as FACS or panning using antibodies.

The PUFAs produced using the subject methods and compositions may
30 be found in the host plant tissue and/or plant part as free fatty acids or in conjugated forms such as acylglycerols, phospholipids, sulfolipids or

glycolipids, and may be extracted from the host cell through a variety of means well-known in the art. Such means may include extraction with organic solvents, sonication, supercritical fluid extraction using for example carbon dioxide, and physical means such as presses, or combinations thereof. Of
5 particular interest is extraction with hexane or methanol and chloroform. Where desirable, the aqueous layer can be acidified to protonate negatively charged moieties and thereby increase partitioning of desired products into the organic layer. After extraction, the organic solvents can be removed by evaporation under a stream of nitrogen. When isolated in conjugated forms, the products are
10 enzymatically or chemically cleaved to release the free fatty acid or a less complex conjugate of interest, and are then subjected to further manipulations to produce a desired end product. Desirably, conjugated forms of fatty acids are cleaved with potassium hydroxide.

PURIFICATION OF FATTY ACIDS

15 If further purification is necessary, standard methods can be employed. Such methods include extraction, treatment with urea, fractional crystallization, HPLC, fractional distillation, silica gel chromatography, high speed centrifugation or distillation, or combinations of these techniques. Protection of reactive groups, such as the acid or alkenyl groups, may be done at any step
20 through known techniques, for example alkylation or iodination. Methods used include methylation of the fatty acids to produce methyl esters. Similarly, protecting groups may be removed at any step. Desirably, purification of fractions containing ARA, DHA and EPA is accomplished by treatment with urea and/or fractional distillation.

USES OF FATTY ACIDS

25 The uses of the fatty acids of subject invention are several. Probes based on the DNAs of the present invention may find use in methods for isolating related molecules or in methods to detect organisms expressing desaturases. When used as probes, the DNAs or oligonucleotides need to be detectable. This
30 is usually accomplished by attaching a label either at an internal site, for

example via incorporation of a modified residue, or at the 5' or 3' terminus. Such labels can be directly detectable, can bind to a secondary molecule that is detectably labeled, or can bind to an unlabelled secondary molecule and a detectably labeled tertiary molecule; this process can be extended as long as is practical to achieve a satisfactorily detectable signal without unacceptable levels of background signal. Secondary, tertiary, or bridging systems can include use of antibodies directed against any other molecule, including labels or other antibodies, or can involve any molecules which bind to each other, for example a biotin-streptavidin/avidin system. Detectable labels typically include radioactive isotopes, molecules which chemically or enzymatically produce or alter light, enzymes which produce detectable reaction products, magnetic molecules, fluorescent molecules or molecules whose fluorescence or light-emitting characteristics change upon binding. Examples of labelling methods can be found in USPN 5,011,770. Alternatively, the binding of target molecules can be directly detected by measuring the change in heat of solution on binding of probe to target via isothermal titration calorimetry, or by coating the probe or target on a surface and detecting the change in scattering of light from the surface produced by binding of target or probe, respectively, as may be done with the BIAcore system.

PUFAs of the subject invention produced by recombinant means find applications in a wide variety of areas. Supplementation of humans or animals with PUFAs in various forms can result in increased levels not only of the added PUFAs, but of their metabolic progeny as well. For example, where the inherent $\Delta 6$ -desaturase pathway is dysfunctional in an individual, treatment with GLA can result not only in increased levels of GLA, but also of downstream products such as ARA and prostaglandins (see Figure 1). Complex regulatory mechanisms can make it desirable to combine various PUFAs, or to add different conjugates of PUFAs, in order to prevent, control or overcome such mechanisms to achieve the desired levels of specific PUFAs in an individual.

PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary supplements, particularly in infant formulas, for patients

undergoing intravenous feeding or for preventing or treating malnutrition. Particular fatty acids such as EPA are used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. The predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-
5 palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 % as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to
10 about 1.04 % as GLA. A preferred ratio of GLA:DGLA:ARA in infant formulas is from about 1:1:4 to about 1:1:1, respectively. Amounts of oils providing these ratios of PUFA can be determined without undue experimentation by one of skill in the art. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or
15 milk fatty acid composition to one more desirable for human or animal consumption.

NUTRITIONAL COMPOSITIONS

The present invention also includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or
20 preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least
25 one oil or acid produced in accordance with the present invention and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults
30 having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to
5 glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey , electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to
10 the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present
15 invention will of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, and rehydration
20 compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

Nutritional Compositions

25 A typical nutritional composition of the present invention will contain edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amounts of such ingredients will vary depending on whether the formulation is intended for use with normal, healthy individuals temporarily exposed to stress, or to subjects having specialized needs due to certain chronic
30 or acute disease states (e.g., metabolic disorders). It will be understood by

persons skilled in the art that the components utilized in a nutritional formulation of the present invention are of semi-purified or purified origin. By semi-purified or purified is meant a material that has been prepared by purification of a natural material or by synthesis. These techniques are well known in the art (See, e.g., Code of Federal Regulations for Food Ingredients and Food Processing; Recommended Dietary Allowances, 10th Ed., National Academy Press, Washington, D.C., 1989).

In a preferred embodiment, a nutritional formulation of the present invention is an enteral nutritional product, more preferably an adult or child enteral nutritional product. Accordingly in a further aspect of the invention, a nutritional formulation is provided that is suitable for feeding adults or children who are experiencing stress. The formula comprises, in addition to the PUFAs of the invention; macronutrients, vitamins and minerals in amounts designed to provide the daily nutritional requirements of adults.

The macronutritional components include edible fats, carbohydrates and proteins. Exemplary edible fats are coconut oil, soy oil, and mono- and diglycerides and the PUFA oils of this invention. Exemplary carbohydrates are glucose, edible lactose and hydrolyzed cornstarch. A typical protein source would be soy protein, electrodialysed whey or electrodialysed skim milk or milk whey, or the hydrolysates of these proteins, although other protein sources are also available and may be used. These macronutrients would be added in the form of commonly accepted nutritional compounds in amount equivalent to those present in human milk or an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid and enteral nutritional formulas are well known in the art and are described in detail in the examples.

The enteral formula can be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or a powder. The powder can be prepared by spray drying the enteral formula prepared as indicated above, and the formula can be reconstituted by rehydrating the concentrate. Adult and infant nutritional formulas are well known in the art and commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum®

from Ross Products Division, Abbott Laboratories). An oil or acid of the present invention can be added to any of these formulas in the amounts described below.

5 The energy density of the nutritional composition when in liquid form, can typically range from about 0.6 Kcal to 3 Kcal per ml. When in solid or powdered form, the nutritional supplement can contain from about 1.2 to more than 9 Kcals per gm, preferably 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and more preferably less than 660 mOsm.

10 The nutritional formula would typically include vitamins and minerals, in addition to the PUFAs of the invention, in order to help the individual ingest the minimum daily requirements for these substances. In addition to the PUFAs listed above, it may also be desirable to supplement the nutritional composition with zinc, copper, and folic acid in addition to antioxidants. It is believed that
15 these substances will also provide a boost to the stressed immune system and thus will provide further benefits to the individual. The presence of zinc, copper or folic acid is optional and is not required in order to gain the beneficial effects on immune suppression. Likewise a pharmaceutical composition can be supplemented with these same substances as well.

20 In a more preferred embodiment, the nutritional contains, in addition to the antioxidant system and the PUFA component, a source of carbohydrate wherein at least 5 weight % of said carbohydrate is an indigestible oligosaccharide. In yet a more preferred embodiment, the nutritional composition additionally contains protein, taurine and carnitine.

25 The PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary substitutes, or supplements, particularly infant formulas, for patients undergoing intravenous feeding or for preventing or treating malnutrition. Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 %
30 as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to about 1.04 % as GLA.

Additionally, the predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Thus, fatty acids such as ARA, DGLA, GLA and/or EPA produced by the invention can be used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. In particular, an oil composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of ARA, DGLA and GLA. More preferably the oil will comprise from about 0.3 to 30% ARA, from about 0.2 to 30% DGLA, and from about 0.2 to about 30% GLA.

In addition to the concentration, the ratios of ARA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, an oil composition which contains two or more of ARA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of ARA:DGLA:DGL ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to ARA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to ARA can be used to produce an ARA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an ARA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of desaturase expression as described can be used to modulate the PUFA levels and ratios. Depending on the expression system used, e.g., cell culture or an animal expressing oil(s) in its milk, the oils also can be isolated and recombined in the desired concentrations and ratios. Amounts of oils providing these ratios of PUFA can be determined following standard protocols. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

For dietary supplementation, the purified PUFAs, or derivatives thereof, may be incorporated into cooking oils, fats or margarines formulated so that in normal use the recipient would receive the desired amount. The PUFAs may also be incorporated into infant formulas, nutritional supplements or other food products, and may find use as anti-inflammatory or cholesterol lowering agents.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream.

Possible routes of administration include, for example, oral, rectal and parenteral. The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Pharmaceutical compositions may be utilized to administer the PUFA component to an individual. Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile solutions or dispersions for ingestion. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances, and the like.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs of the invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with the antioxidants and the PUFA component. The amount of the antioxidants and PUFA component that should

be incorporated into the pharmaceutical formulation should fit within the guidelines discussed above.

As used in this application, the term "treat" refers to either preventing, or reducing the incidence of, the undesired occurrence. For example, to treat
5 immune suppression refers to either preventing the occurrence of this suppression or reducing the amount of such suppression. The terms "patient" and "individual" are being used interchangeably and both refer to an animal. The term "animal" as used in this application refers to any warm-blooded mammal including, but not limited to, dogs, humans, monkeys, and apes. As
10 used in the application the term "about" refers to an amount varying from the stated range or number by a reasonable amount depending upon the context of use. Any numerical number or range specified in the specification should be considered to be modified by the term about.

"Dose" and "serving" are used interchangeably and refer to the amount
15 of the nutritional or pharmaceutical composition ingested by the patient in a single setting and designed to deliver effective amounts of the antioxidants and the structured triglyceride. As will be readily apparent to those skilled in the art, a single dose or serving of the liquid nutritional powder should supply the amount of antioxidants and PUFAs discussed above. The amount of the dose or
20 serving should be a volume that a typical adult can consume in one sitting. This amount can vary widely depending upon the age, weight, sex or medical condition of the patient. However as a general guideline, a single serving or dose of a liquid nutritional produce should be considered as encompassing a volume from 100 to 600 ml, more preferably from 125 to 500 ml and most
25 preferably from 125 to 300 ml.

The PUFAs of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils,
30 cooking oils, cooking fats, meats, fish and beverages.

Pharmaceutical Applications

For pharmaceutical use (human or veterinary), the compositions are generally administered orally but can be administered by any route by which they may be successfully absorbed, e.g., parenterally (i.e. subcutaneously, intramuscularly or intravenously), rectally or vaginally or topically, for example, as a skin ointment or lotion. The PUFAs of the present invention may be administered alone or in combination with a pharmaceutically acceptable carrier or excipient. Where available, gelatin capsules are the preferred form of oral administration. Dietary supplementation as set forth above also can provide an oral route of administration. The unsaturated acids of the present invention may be administered in conjugated forms, or as salts, esters, amides or prodrugs of the fatty acids. Any pharmaceutically acceptable salt is encompassed by the present invention; especially preferred are the sodium, potassium or lithium salts. Also encompassed are the N-alkylpolyhydroxamine salts, such as N-methyl glucamine, found in PCT publication WO 96/33155. The preferred esters are the ethyl esters. As solid salts, the PUFAs also can be administered in tablet form. For intravenous administration, the PUFAs or derivatives thereof may be incorporated into commercial formulations such as Intralipids. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of ARA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered, either alone or in mixtures with other PUFAs, to achieve a normal fatty acid profile in a patient. Where desired, the individual components of formulations may be individually provided in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g, or even 100 g daily, and is preferably from 10 mg to 1, 2, 5 or 10 g daily as required, or molar equivalent amounts of derivative forms thereof. Parenteral nutrition compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention; preferred is a composition having from about 1 to about 25 weight percent of the total PUFA composition as GLA (USPN 5,196,198). Other vitamins, and particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. Where desired, a

preservative such as α tocopherol may be added, typically at about 0.1% by weight.

Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectible solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances and the like.

An especially preferred pharmaceutical composition contains diacetyltartaric acid esters of mono- and diglycerides dissolved in an aqueous medium or solvent. Diacetyltartaric acid esters of mono- and diglycerides have an HLB value of about 9-12 and are significantly more hydrophilic than existing antimicrobial lipids that have HLB values of 2-4. Those existing hydrophobic lipids cannot be formulated into aqueous compositions. As disclosed herein, those lipids can now be solubilized into aqueous media in combination with diacetyltartaric acid esters of mono-and diglycerides. In accordance with this embodiment, diacetyltartaric acid esters of mono- and diglycerides (e.g., DATEM-C12:0) is melted with other active antimicrobial lipids (e.g., 18:2 and 12:0 monoglycerides) and mixed to obtain a homogeneous mixture.

Homogeneity allows for increased antimicrobial activity. The mixture can be completely dispersed in water. This is not possible without the addition of diacetyltartaric acid esters of mono- and diglycerides and premixing with other monoglycerides prior to introduction into water. The aqueous composition can
5 then be admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants as may be required to form a spray or inhalant.

The present invention also encompasses the treatment of numerous disorders with fatty acids. Supplementation with PUFAs of the present
10 invention can be used to treat restenosis after angioplasty. Symptoms of inflammation, rheumatoid arthritis, and asthma and psoriasis can be treated with the PUFAs of the present invention. Evidence indicates that PUFAs may be involved in calcium metabolism, suggesting that PUFAs of the present invention may be used in the treatment or prevention of osteoporosis and of
15 kidney or urinary tract stones.

The PUFAs of the present invention can be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions; addition of fatty acids has been shown to slow their growth and cause cell death, and to increase their susceptibility to chemotherapeutic agents.
20 GLA has been shown to cause reexpression on cancer cells of the E-cadherin cellular adhesion molecules, loss of which is associated with aggressive metastasis. Clinical testing of intravenous administration of the water soluble lithium salt of GLA to pancreatic cancer patients produced statistically significant increases in their survival. PUFA supplementation may also be
25 useful for treating cachexia associated with cancer.

The PUFAs of the present invention can also be used to treat diabetes (USPN 4,826,877; Horrobin *et al.*, Am. J. Clin. Nutr. Vol. 57 (Suppl.), 732S-737S). Altered fatty acid metabolism and composition has been demonstrated in diabetic animals. These alterations have been suggested to be involved in
30 some of the long-term complications resulting from diabetes, including retinopathy, neuropathy, nephropathy and reproductive system damage.

Primrose oil, which contains GLA, has been shown to prevent and reverse diabetic nerve damage.

The PUFAs of the present invention can be used to treat eczema, reduce blood pressure and improve math scores. Essential fatty acid deficiency has
5 been suggested as being involved in eczema, and studies have shown beneficial effects on eczema from treatment with GLA. GLA has also been shown to reduce increases in blood pressure associated with stress, and to improve performance on arithmetic tests. GLA and DGLA have been shown to inhibit platelet aggregation, cause vasodilation, lower cholesterol levels and inhibit
10 proliferation of vessel wall smooth muscle and fibrous tissue (Brenner *et al.*, Adv. Exp. Med. Biol. Vol. 83, p. 85-101, 1976). Administration of GLA or DGLA, alone or in combination with EPA, has been shown to reduce or prevent gastro-intestinal bleeding and other side effects caused by non-steroidal anti-inflammatory drugs (USPN 4,666,701). GLA and DGLA have also been shown
15 to prevent or treat endometriosis and premenstrual syndrome (USPN 4,758,592) and to treat myalgic encephalomyelitis and chronic fatigue after viral infections (USPN 5,116,871).

Further uses of the PUFAs of this invention include use in treatment of AIDS, multiple sclerosis, acute respiratory syndrome, hypertension and
20 inflammatory skin disorders. The PUFAs of the inventions also can be used for formulas for general health as well as for geriatric treatments.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals, as well as
25 humans, as animals experience many of the same needs and conditions as human. For example, the oil or acids of the present invention may be utilized in animal feed supplements or as animal feed substitutes.

The following examples are presented by way of illustration, not of limitation.

Examples

- 5 Example 1 Isolation of $\Delta 5$ Desaturase Nucleotide Sequence from
 Mortierella alpina
- Example 2 Isolation of $\Delta 6$ Desaturase Nucleotide Sequence from
 Mortierella alpina
- Example 3 Identification of $\Delta 6$ Desaturases Homologues to the
 Mortierella alpina Δ Desaturase
- Example 4 Isolation of D-12 Desaturase Nucleotide Sequence from
 Mortierella alpina
- 10 Example 5 Isolation of Cytochrome b5 Reductase Nucleotide
 Sequence from *Mortierella alpina*
- Example 6 Expression of *M. alpina* Desaturase Clones in Baker's
 Yeast
- Example 7 Fatty Acid Analysis of Leaves from Ma29 Transgenic
15 *Brassica* Plants
- Example 8 Expression of *M. alpina* $\Delta 6$ Desaturase in *Brassica*
 napus
- Example 9 Expression of *M. alpina* $\Delta 12$ desaturase in *Brassica*
 napus
- 20 Example 10 Simultaneous expression of *M. alpina* $\Delta 6$ and $\Delta 12$
 desaturases in *Brassica napus*
- Example 11 Simultaneous expression of *M. alpina* $\Delta 5$ and $\Delta 6$
 desaturases in *Brassica napus*
- Example 12 Simultaneous expression of *M. alpina* $\Delta 5$, $\Delta 6$ and $\Delta 12$
25 desaturases in *Brassica napus*
- Example 13 Stereospecific Distribution of $\Delta 6$ -Desaturated Oils
- Example 14 Fatty Acid Compositions of Transgenic Plants

Example 15 Combined Expression of $\Delta 6$ and $\Delta 12$ Desaturases in *B. napus* Achieved by Crossing

Example 16 Expression of *M. alpina* desaturases in soybean

Example 17 Human Desaturase Gene Sequences

5

Example 1

Isolation of a $\Delta 5$ -desaturase Nucleotide Sequence from *Mortierella alpina*

Mortierella alpina produces arachidonic acid (ARA, 20:4) from the precursor 20:3 by a $\Delta 5$ -desaturase. A nucleotide sequence encoding the $\Delta 5$ -desaturase from *Mortierella alpina* (see Figure 7) was obtained through PCR
10 amplification using *M. alpina* 1st strand cDNA and degenerate oligonucleotide primers corresponding to amino acid sequences conserved between $\Delta 6$ -desaturases from *Synechocystis* and *Spirulina*. The procedure used was as follows:

Total RNA was isolated from a 3 day old PUFA-producing culture of
15 *Mortierella alpina* using the protocol of Hoge *et al.* (1982) *Experimental Mycology* 6:225-232. The RNA was used to prepare double-stranded cDNA using BRL's lambda-ZipLox system, following the manufacturer's instructions. Several size fractions of the *M. alpina* cDNA were packaged separately to yield libraries with different average-sized inserts. The "full-length" library contains
20 approximately 3×10^6 clones with an average insert size of 1.77 kb. The "sequencing-grade" library contains approximately 6×10^5 clones with an average insert size of 1.1 kb.

5 μ g of total RNA was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTTTT-
25 3' (SEQ ID NO:19.) Degenerate oligonucleotides were designed to regions conserved between the two cyanobacterial $\Delta 6$ -desaturase sequences. The specific primers used were:

D6DESAT-F3 (SEQ ID NO:20)

5'-CUACUACUACUACAYCAYACOTAYACOAAYAT-3'

D6DESAT-R3 (SEQ ID NO:21)

5'-CAUCAUCAUCAUOGGAAOARRTGRTG-3'

- 5 where Y=C+T, R=A+G, and O=I+C. PCR amplification was carried out in a 25 μ l volume containing: template derived from 40 ng total RNA, 2 pM each primer, 200 μ M each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH₄)₂SO₄, 2 mM MgCl₂. Samples were subjected to an initial desaturation step of 95 degrees (all temperatures Celsius) for 5 minutes, then
- 10 held at 72 degrees while 0.2 U of Taq polymerase were added. PCR thermocycling conditions were as follows: 94 degrees for 1 min., 45 degrees for 1.5 min., 72 degrees for 2 min. PCR was continued for 35 cycles. PCR using these primers on the *M. alpina* first-strand cDNA produced a 550 bp reaction product. Comparison of the deduced amino acid sequence of the *M.*
- 15 *alpina* PCR fragment revealed regions of homology with Δ 6-desaturases (see Figure 4). However, there was only about 28% identity over the region compared. The deduced amino acid sequence is presented in SEQ ID NO:14.

- The PCR product was used as a probe to isolate corresponding cDNA clones from a *M. alpina* library. The longest cDNA clone, Ma29, was
- 20 designated pCGN5521 and has been completely sequenced on both strands. The cDNA is contained as a 1481 bp insert in the vector pZL1 (Bethesda Research Laboratories) and, beginning with the first ATG, contains an open reading frame encoding 446 amino acids. The reading frame contains the sequence deduced from the PCR fragment. The sequence of the cDNA insert
- 25 was found to contain regions of homology to Δ 6-desaturases (see Figure 8). For example, three conserved "histidine boxes" (that have been observed in other membrane-bound desaturases (Okuley *et al.*, (1994) *The Plant Cell* 6:147-158)) were found to be present in the *Mortierella* sequence at amino acid positions 171-175, 207-212, and 387-391 (see Figure 5A-5D). However, the typical
- 30 "HXXHH" amino acid motif for the third histidine box for the *Mortierella*

desaturase was found to be QXXHH. The amino-terminus of the encoded protein, showed significant homology to cytochrome b5 proteins. Thus, the *Mortierella* cDNA clone appears to represent a fusion between a cytochrome b5 and a fatty acid desaturase. Since cytochrome b5 is believed to function as the electron donor for membrane-bound desaturase enzymes, it is possible that the N-terminal cytochrome b5 domain of this desaturase protein is involved in its function. This may be advantageous when expressing the desaturase in heterologous systems for PUFA production.

Example 2

10 Isolation of $\Delta 6$ Desaturase Nucleotide Sequence from *Mortierella alpina*

A nucleic acid sequence from a partial cDNA clone, Ma524, encoding a $\Delta 6$ fatty acid desaturase from *Mortierella alpina* was obtained by random sequencing of clones from the *M. alpina* cDNA library described in Example 1. cDNA-containing plasmids were excised as follows:

15 Five μ l of phage were combined with 100 μ l of *E. coli* DH10B(ZIP) grown in ECLB plus 10 μ g/ml kanamycin, 0.2% maltose, and 10 mM $MgSO_4$ and incubated at 37 degrees for 15 minutes. 0.9 ml SOC was added and 100 μ l of the bacteria immediately plated on each of 10 ECLB + 50 μ g Pen plates. No 45 minute recovery time was needed. The plates were incubated overnight at 37
20 degrees. Colonies were picked into ECLB + 50 μ g Pen media for overnight cultures to be used for making glycerol stocks and miniprep DNA. An aliquot of the culture used for the miniprep is stored as a glycerol stock. Plating on ECLB + 50 μ g Pen/ml resulted in more colonies and a greater proportion of colonies containing inserts than plating on 100 μ g/ml Pen.

25 Random colonies were picked and plasmid DNA purified using Qiagen miniprep kits. DNA sequence was obtained from the 5' end of the cDNA insert and compared to the databases using the BLAST algorithm. Ma524 was identified as a putative $\Delta 6$ desaturase based on DNA sequence homology to previously identified $\Delta 6$ desaturases. A full-length cDNA clone was isolated

from the *M. alpina* library. The abundance of this clone appears to be slightly (2X) less than Ma29. Ma524 displays significant homology to a portion of a *Caenorhabditis elegans* cosmid, WO6D2.4, a cytochrome b5/desaturase fusion protein from sunflower, and the two $\Delta 6$ desaturases in the public databanks
5 those from *Synechocystis* and *Spirulina*.

In addition, Ma524 shows significant homology to the borage $\Delta 6$ -desaturase sequence (PCT publication WO 96/21022). Ma524 thus appears to encode a $\Delta 6$ -desaturase that is related to the borage and algal $\Delta 6$ -desaturases. It should be noted that, although the amino acid sequences of Ma524 and the
10 borage $\Delta 6$ are similar, the base composition of the cDNAs is quite different: the borage cDNA has an overall base composition of 60 % A+T, with some regions exceeding 70 %, while Ma524 has an average of 44 % A+T base composition, with no regions exceeding 60 %. This may have implications for expressing the cDNAs in microorganisms or animals which favor different base compositions.
15 It is known that poor expression of recombinant genes can occur when the host has a very different base composition from that of the introduced gene. Speculated mechanisms for such poor expression include decreased stability or translatability of the mRNA.

Example 3

20 Identification of $\Delta 6$ -desaturases Homologous to the *Mortierella alpina* $\Delta 6$ -desaturase

Nucleic acid sequences that encode putative $\Delta 6$ -desaturases were identified through a BLASTX search of the est databases through NCBI using the Ma524 amino acid sequence. Several sequences showed significant
25 homology. In particular, the deduced amino acid sequence of two *Arabidopsis thaliana* sequences, (accession numbers F13728 and T42806) showed homology to two different regions of the deduced amino acid sequence of Ma524. The following PCR primers were designed: ATTS4723-FOR (complementary to F13728) 5'-CUACUACUACUAGGAGTCCTCTA
30 CGGTGTTTTG, SEQ ID NO:22, and T42806-REV (complementary to

T42806) 5' CAUCAUCAUATGATGCTCAAGCTGAACTG, SEQ ID NO:23. Five µg of total RNA isolated from developing siliques of *Arabidopsis thaliana* was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CCAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTTTT-3', (SEQ ID NO:24). PCR was carried out in a 50 µl volume containing: template derived from 25 ng total RNA, 2 pM each primer, 200 µM each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.2 U Taq Polymerase. Cycle conditions were as follows: 94 degrees for 30 sec., 50 degrees for 30 sec., 72 degrees for 30 sec. PCR was continued for 35 cycles followed by an additional extension at 72 degrees for 7 minutes. PCR resulted in a fragment of ~750 base pairs which was subsequently subcloned, named 12-5, and sequenced. Each end of this fragment corresponds to the *Arabidopsis* est from which the PCR primers were derived. This is the sequence named 12-5. The deduced amino acid sequence of 12-5 is compared to that of Ma524 and ests from human (W28140), mouse (W53753), and *C. elegans* (R05219) in Figure 4. Based on homology, these sequences represent desaturase polypeptides. The full-length genes can be cloned using probes based on the est sequences. The genes can then be placed in expression vectors and expressed in host cells and their specific Δ⁶- or other desaturase activity can be determined as described below.

Example 4

Isolation of Δ-12 Desaturase Nucleotide Sequence from *Mortierella alpina*

Based on the fatty acids it accumulates, *Mortierella alpina* has an ω₆ type desaturase. The ω₆ desaturase is responsible for the production of linoleic acid (18:2) from oleic acid (18:1). Linoleic acid (18:2) is a substrate for a Δ⁶ desaturase. This experiment was designed to determine if *Mortierella alpina* has a Δ¹²-desaturase polypeptide, and if so, to identify the corresponding nucleotide sequence. A random colony from the *M. alpina* sequencing grade library, Ma648, was sequenced and identified as a putative desaturase based on DNA sequence homology to previously identified desaturases, as described for

Ma524 (*see* Example 2). The deduced amino acid sequence from the 5' end of the Ma648 cDNA displays significant homology to soybean microsomal ω 6 (Δ 12) desaturase (accession #L43921) as well as castor bean oleate 12-hydroxylase (accession #U22378). In addition, homology is observed to a variety of other ω 6 (Δ 12) and ω 3 (Δ 15) fatty acid desaturase sequences.

Example 5

Isolation of Cytochrome b5 Reductase Nucleotide Sequence from *Mortierella alpina*

A nucleic acid sequence encoding a cytochrome b5 reductase from *Mortierella alpina* was obtained as follows. A cDNA library was constructed based on total RNA isolated from *Mortierella alpina* as described in Example 1. DNA sequence was obtained from the 5' and 3' ends of one of the clones, M12-27. A search of public databanks with the deduced amino acid sequence of the 3' end of M12-27 (*see* Figure 5) revealed significant homology to known cytochrome b5 reductase sequences. Specifically, over a 49 amino acid region, the *Mortierella* clone shares 55% identity (73% homology) with a cytochrome b5 reductase from pig (*see* Figure 4).

Example 6

Expression of *M. alpina* Desaturase Clones in Baker's Yeast **Yeast Transformation**

Lithium acetate transformation of yeast was performed according to standard protocols (*Methods in Enzymology*, Vol. 194, p. 186-187, 1991). Briefly, yeast were grown in YPD at 30°C. Cells were spun down, resuspended in TE, spun down again, resuspended in TE containing 100 mM lithium acetate, spun down again, and resuspended in TE/lithium acetate. The resuspended yeast were incubated at 30°C for 60 minutes with shaking. Carrier DNA was added, and the yeast were aliquoted into tubes. Transforming DNA was added, and the tubes were incubated for 30 min. at 30°C. PEG solution (35% (w/v) PEG 4000, 100 mM lithium acetate, TE pH7.5) was added followed by a 50

min. incubation at 30°C. A 5 min. heat shock at 42°C was performed, the cells were pelleted, washed with TE, pelleted again and resuspended in TE. The resuspended cells were then plated on selective media.

Desaturase Expression in Transformed Yeast

5 cDNA clones from *Mortierella alpina* were screened for desaturase activity in baker's yeast. A canola $\Delta 15$ -desaturase (obtained by PCR using 1st strand cDNA from *Brassica napus* cultivar 212/86 seeds using primers based on the published sequence (Arondel *et al. Science* 258:1353-1355)) was used as a positive control. The $\Delta 15$ -desaturase gene and the gene from cDNA clone
10 Ma29 was put in the expression vector pYES2 (Invitrogen), resulting in plasmids pCGR-2 and pCGR-4, respectively. These plasmids were transfected into *S. cerevisiae* yeast strain 334 and expressed after induction with galactose and in the presence of substrates that allowed detection of specific desaturase activity. The control strain was *S. cerevisiae* strain 334 containing the unaltered
15 pYES2 vector. The substrates used, the products produced and the indicated desaturase activity were: DGLA (conversion to ARA would indicate $\Delta 5$ -desaturase activity), linoleic acid (conversion to GLA would indicate $\Delta 6$ -desaturase activity; conversion to ALA would indicate $\Delta 15$ -desaturase activity), oleic acid (an endogenous substrate made by *S. cerevisiae*, conversion to
20 linoleic acid would indicate $\Delta 12$ -desaturase activity, which *S. cerevisiae* lacks), or ARA (conversion to EPA would indicate $\Delta 17$ -desaturase activity). The results are provided in Table 1 below. The lipid fractions were extracted as follows: Cultures were grown for 48-52 hours at 15°C. Cells were pelleted by centrifugation, washed once with sterile ddH₂O, and repelleted. Pellets were
25 vortexed with methanol; chloroform was added along with tritridecanoin (as an internal standard). The mixtures were incubated for at least one hour at room temperature or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated
30 at 40°C under a stream of nitrogen. The extracted lipids were then derivatized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by

adding 2 ml of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C to 100°C for 30 minutes and cooled to room temperature. Approximately 2 ml of 14 % boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml
5 of water and 1 ml of hexane were added to extract the FAME for analysis by GC. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate added) and then multiplying by 100. To calculate the oleic acid percent conversion, as no
10 substrate was added, the total linoleic acid produced was divided by the sum of (oleic acid and linoleic acid produced), then multiplying by 100.

Table 1***M. alpina* Desaturase Expression in Baker's Yeast**

| CLONE | TYPE OF ENZYME ACTIVITY | % CONVERSION OF SUBSTRATE |
|------------------------------------|----------------------------|--------------------------------|
| pCGR-2 | $\Delta 6$ | 0 (18:2 to 18:3 ω 6) |
| (canola $\Delta 15$ desaturase) | $\Delta 15$ | 16.3 (18:2 to 18:3 ω 3) |
| | $\Delta 5$ | 2.0 (20:3 to 20:4 ω 6) |
| | $\Delta 17$ | 2.8 (20:4 to 20:5 ω 3) |
| | $\Delta 12$ | 1.8 (18:1 to 18:2 ω 6) |
| pCGR-4 | $\Delta 6$ | 0 |
| (M. alpina | $\Delta 15$ | 0 |
| $\Delta 6$ -like, Ma29) | $\Delta 5$ | 15.3 |
| | $\Delta 17$ | 0.3 |
| | $\Delta 12$ | 3.3 |
| pCGR-7 | $\Delta 6$ | 0 |
| (M. alpina | $\Delta 15$ | 3.8 |
| $\Delta 12$ -like, Ma648 | $\Delta 5$ | 2.2 |
| | $\Delta 17$ | 0 |
| | $\Delta 12$ | 63.4 |

5 The $\Delta 15$ -desaturase control clone exhibited 16.3% conversion of the
 substrate. The pCGR-4 clone expressing the Ma29 cDNA converted 15.3% of
 the 20:3 substrate to 20:4 ω 6, indicating that the gene encodes a $\Delta 5$ -desaturase.
 The background (non-specific conversion of substrate) was between 0-3% in
 these cases. The pCGR-5 clone expressing the Ma524 cDNA showed 6%
 conversion of the substrate to GLA, indicating that the gene encodes a $\Delta 6$ -
 10 desaturase. The pCGR-7 clone expressing the Ma648 cDNA converted 63.4%
 conversion of the substrate to LA, indicating that the gene encodes a $\Delta 12$ -
 desaturase. Substrate inhibition of activity was observed by using different
 concentrations of the substrate. When substrate was added to 100 μ M, the
 percent conversion to product dropped as compared to when substrate was
 15 added to 25 μ M (see below). These data show that desaturases with different

substrate specificities can be expressed in a heterologous system and used to produce PUFAs.

Table 2 represents fatty acids of interest as a percent of the total lipid extracted from the yeast host *S. cerevisiae* 334 with the indicated plasmid. No
5 glucose was present in the growth media. Affinity gas chromatography was used to separate the respective lipids. GC/MS was employed to verify the identity of the product(s). The expected product for the *B. napus* $\Delta 15$ -desaturase, α -linolenic acid, was detected when its substrate, linoleic acid, was added exogenously to the induced yeast culture. This finding demonstrates that
10 yeast expression of a desaturase gene can produce functional enzyme and detectable amounts of product under the current growth conditions. Both exogenously added substrates were taken up by yeast, although slightly less of the longer chain PUFA, dihomo- γ -linolenic acid (20:3), was incorporated into yeast than linoleic acid (18:2) when either was added in free form to the induced
15 yeast cultures. γ -linolenic acid was detected when linoleic acid was present during induction and expression of *S. cerevisiae* 334 (pCGR-5). The presence of this PUFA demonstrates $\Delta 6$ -desaturase activity from pCGR-5 (MA524). Linoleic acid, identified in the extracted lipids from expression of *S. cerevisiae* 334 (pCGR-7), classifies the cDNA MA648 from *M. alpina* as the $\Delta 12$ -
20 desaturase.

Table 2
Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

| Plasmid in Yeast (enzyme) | 18:2 Incorporated | α -18:3 Produced | γ -18:3 Produced | 20:3 Incorporated | 20:4 Produced | 18:1* Present | 18:2 Produced |
|---------------------------------|----------------------|----------------------------|----------------------------|----------------------|------------------|------------------|------------------|
| pYES2 (control) | 66.9 | 0 | 0 | 58.4 | 0 | 4 | 0 |
| pCGR-2 (Δ 15) | 60.1 | 5.7 | 0 | 50.4 | 0 | 0.7 | 0 |
| pCGR-4 (Δ 5) | 67 | 0 | 0 | 32.3 | 5.8 | 0.8 | 0 |
| pCGR-5 (Δ 6) | 62.4 | 0 | 4.0 | 49.9 | 0 | 2.4 | 0 |
| pCGR-7 (Δ 12) | 65.6 | 0 | 0 | 45.7 | 0 | 7.1 | 12.2 |

100 μ M substrate added

* 18:1 is an endogenous fatty acid in yeast

- 5 Key To Tables
18:1 =oleic acid
18:2 =linoleic acid
 α -18:3 = α -linolenic acid
 γ -18:3 = γ -linolenic acid
18:4 =stearidonic acid
20:3 =dihomo- γ -linolenic acid
20:4 =arachidonic acid

Example 7

Expression of $\Delta 5$ Desaturase in Plants

Expression in Leaves

This experiment was designed to determine whether leaves expressing
5 Ma29 (as determined by Northern) were able to convert exogenously applied
DGLA (20:3) to ARA (20:4).

The Ma29 desaturase cDNA was modified by PCR to introduce
convenient restriction sites for cloning. The desaturase coding region has been
inserted into a d35 cassette under the control of the double 35S promoter for
10 expression in *Brassica* leaves (pCGN5525) following standard protocols (*see*
USPN 5,424,200 and USPN 5,106,739). Transgenic *Brassica* plants containing
pCGN5525 were generated following standard protocols (*see* USPN 5,188,958
and USPN 5,463,174).

In the first experiment, three plants were used: a control, LPO04-1, and
15 two transgenics,, 5525-23 and 5525-29. LP004 is a low-linolenic *Brassica*
variety. Leaves of each were selected for one of three treatments: water, GLA
or DGLA. GLA and DGLA were purchased as sodium salts from NuChek Prep
and dissolved in water at 1 mg/ml. Aliquots were capped under N₂ and stored at
-70 degrees C. Leaves were treated by applying a 50 μ l drop to the upper
20 surface and gently spreading with a gloved finger to cover the entire surface.
Applications were made approximately 30 minutes before the end of the light
cycle to minimize any photo-oxidation of the applied fatty acids. After 6 days
of treatment one leaf from each treatment was harvested and cut in half through
the mid rib. One half was washed with water to attempt to remove
25 unincorporated fatty acid. Leaf samples were lyophilized overnight, and fatty
acid composition determined by gas chromatography (GC). The results are
shown in Table 3.

Table 3
Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants

| Treatment | SPL | 16:00 | 16:01 | 18:00 | 18:01 | 18:10 | 18:1v | 18:02 | 18:3g | 18:03 | 18:04 | 20:00 | 20:01 |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | # | % | % | % | % | % | % | % | % | % | % | % | % |
| Water | 33 | 12.95 | 0.08 | 2.63 | 2.51 | 1.54 | 0.98 | 16.76 | 0 | 45.52 | 0 | 0.09 | 0 |
| | 34 | 13.00 | 0.09 | 2.67 | 2.56 | 1.55 | 1.00 | 16.86 | 0 | 44.59 | 0 | 0.15 | 0 |
| | 35 | 14.13 | 0.09 | 2.37 | 2.15 | 1.27 | 0.87 | 16.71 | 0 | 49.91 | 0 | 0.05 | 0.01 |
| | 36 | 13.92 | 0.08 | 2.32 | 2.07 | 1.21 | 0.86 | 16.16 | 0 | 50.25 | 0 | 0.05 | 0 |
| | 37 | 13.79 | 0.11 | 2.10 | 2.12 | 1.26 | 0.86 | 15.90 | 0.08 | 46.29 | 0 | 0.54 | 0.01 |
| | 38 | 12.80 | 0.09 | 1.94 | 2.08 | 1.35 | 0.73 | 14.54 | 0.11 | 45.61 | 0 | 0.49 | 0.01 |
| GLA | 39 | 12.10 | 0.09 | 2.37 | 2.10 | 1.29 | 0.82 | 14.85 | 1.63 | 43.66 | 0 | 0.53 | 0 |
| | 40 | 12.78 | 0.10 | 2.34 | 2.22 | 1.36 | 0.86 | 15.29 | 1.72 | 47.22 | 0 | 0.50 | 0.02 |
| | 41 | 13.71 | 0.07 | 2.68 | 2.16 | 1.34 | 0.82 | 15.92 | 2.12 | 46.55 | 0 | 0.09 | 0 |
| | 42 | 14.10 | 0.07 | 2.75 | 2.35 | 1.51 | 0.84 | 16.66 | 1.56 | 46.41 | 0 | 0.09 | 0.01 |
| | 43 | 13.62 | 0.09 | 2.22 | 1.94 | 1.21 | 0.73 | 14.68 | 2.42 | 46.69 | 0 | 0.51 | 0.01 |
| | 44 | 13.92 | 0.09 | 2.20 | 2.17 | 1.32 | 0.85 | 15.22 | 2.30 | 46.05 | 0 | 0.53 | 0.02 |
| DGLA | 45 | 12.45 | 0.14 | 2.30 | 2.28 | 1.37 | 0.91 | 15.65 | 0.07 | 44.62 | 0 | 0.12 | 0.01 |
| | 46 | 12.67 | 0.15 | 2.69 | 2.50 | 1.58 | 0.92 | 15.96 | 0.09 | 42.77 | 0 | 0.56 | 0.01 |
| | 47 | 12.56 | 0.23 | 3.40 | 1.98 | 1.13 | 0.86 | 13.57 | 0.03 | 45.52 | 0 | 0.51 | 0.01 |
| | 48 | 13.07 | 0.24 | 3.60 | 2.51 | 1.63 | 0.88 | 13.54 | 0.04 | 45.13 | 0 | 0.50 | 0.01 |
| | 49 | 13.26 | 0.07 | 2.81 | 2.34 | 1.67 | 0.67 | 16.04 | 0.04 | 43.89 | 0 | 0.59 | 0 |
| | 50 | 13.53 | 0.07 | 2.84 | 2.41 | 1.70 | 0.70 | 16.07 | 0.02 | 44.90 | 0 | 0.60 | 0.01 |

Table 3 - Continued
Fatty Acid Analysis of Leaves from Ma29 Transgenic Brassica Plants

| Treatment | SPL | 20:02 | 20:03 | 20:04 | 20:05 | 22:00 | 22:01 | 22:02 | 22:03 | 22:06 | 24:0 | 24:1 |
|-----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|------|
| | # | % | % | % | % | % | % | % | % | % | % | % |
| Water | 33 | 0 | 0 | 0.29 | 0 | 0.01 | 0.09 | 16.26 | 0 | 0 | 0.38 | 0.18 |
| | 34 | 0.01 | 0 | 0.26 | 0 | 0.14 | 0.10 | 16.82 | 0.02 | 0.05 | 0.36 | 0.27 |
| | 35 | 0.01 | 0 | 0.25 | 0 | 0.12 | 0.06 | 11.29 | 0.04 | 0.05 | 0.29 | 0.25 |
| | 36 | 0 | 0.01 | 0.26 | 0 | 0.07 | 0.04 | 11.82 | 0.03 | 0.36 | 0.28 | 0.21 |
| | 37 | 0.02 | 0 | 0.21 | 0 | 0.18 | 0.08 | 15.87 | 0.06 | 0.20 | 0.30 | 0.17 |
| | 38 | 0.01 | 0 | 0.24 | 0 | 0.15 | 0.07 | 13.64 | 0.09 | 0.08 | 5.89 | 0.23 |
| GLA | 39 | 0.02 | 0.01 | 0.27 | 0 | 0.10 | 0.08 | 16.25 | 3.42 | 0.19 | 0.37 | 0.17 |
| | 40 | 0.01 | 0 | 0.27 | 0 | 0.10 | 0.10 | 14.74 | 0.05 | 0.10 | 0.36 | 0.14 |
| | 41 | 0 | 0 | 0.27 | 0 | 0.20 | 0.10 | 13.15 | 0.13 | 0.29 | 0.33 | 0.20 |
| | 42 | 0 | 0 | 0.28 | 0 | 0.11 | 0.11 | 12.60 | 0.02 | 0.24 | 0.38 | 0.13 |
| | 43 | 0.01 | 0 | 0.28 | 0 | 0.10 | 0.03 | 14.73 | 0.01 | 0.24 | 0.34 | 0.14 |
| | 44 | 0.02 | 0 | 0.26 | 0 | 0.13 | 0.07 | 14.43 | 0.05 | 0.16 | 0.33 | 0.17 |
| DGLA | 45 | 0.06 | 1.21 | 0.26 | 0 | 0.07 | 0.07 | 18.67 | 0.02 | 0.21 | 0.36 | 0.13 |
| | 46 | 0 | 1.94 | 0.27 | 0 | 0.11 | 0.09 | 17.97 | 0.09 | 0.39 | 0.41 | 0.11 |
| | 47 | 0.01 | 0.69 | 0.96 | 0 | 0.11 | 0.07 | 17.96 | 0 | 0.22 | 0.49 | 0.20 |
| | 48 | 0.01 | 0.70 | 0.74 | 0 | 0.14 | 0.09 | 17.14 | 0.05 | 0.32 | 0.52 | 0.10 |
| | 49 | 0 | 0.35 | 1.11 | 0 | 0.10 | 0.07 | 17.26 | 0.07 | 0.23 | 0.39 | 0.18 |
| | 50 | 0 | 0.20 | 0.87 | 0 | 0.21 | 0.07 | 15.73 | 0.04 | 0.15 | 0.37 | 0.18 |

Leaves treated with GLA contained from 1.56 to 2.4 wt% GLA. The fatty acid analysis showed that the lipid composition of control and transgenic leaves was essentially the same. Leaves of control plants treated with DGLA contained 1.2-1.9 w% DGLA and background amounts of ARA (.26-.27 wt%).

- 5 Transgenic leaves contained only .2-.7 wt% DGLA, but levels of ARA were increased (.74-1.1 wt%) indicating that the DGLA was converted to ARA in these leaves.

Expression in Seed

- 10 The purpose of this experiment was to determine whether a construct with the seed specific napin promoter would enable expression in seed.

The Ma29 cDNA was modified by PCR to introduce *XhoI* cloning sites upstream and downstream of the start and stop codons, respectively, using the following primers:

Madxho-forward:

- 15 5'-CUACUACUACUACTCGAGCAAGATGGGAACGGACCAAGG
(SEQ ID NO:25)

Madxho-reverse:

5'-CAUCAUCAUCAUCTCGAGCTACTCTTCCTTGGGACGGAG
(SEQ ID NO:26).

- 20 The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5522 and the $\Delta 5$ desaturase sequence was verified by sequencing of both strands.

- 25 For seed-specific expression, the Ma29 coding region was cut out of pCGN5522 as an *XhoI* fragment and inserted into the *SalI* site of the napin expression cassette, pCGN3223, to create pCGN5528. The *HindIII* fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the *HindIII* site of pCGN1557 to create pCGN5531. Two copies of the napin transcriptional unit were inserted in tandem. This tandem construct can permit higher expression of

the desaturases per genetic loci. pCGN5531 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

The fatty acid composition of twenty-seed pools of mature T2 seeds was analyzed by GC. Table 4 shows the results obtained with independent
5 transformed lines as compared to non-transformed LP004 seed. The transgenic seeds containing pCGN5531 contain two fatty acids that are not present in the control seeds, tentatively identified as taxoleic acid (5,9-18:2) and pinolenic acid (5,9,12-18:3), based on their elution relative to oleic and linoleic acid. These would be the expected products of $\Delta 5$ desaturation of oleic and linoleic
10 acids. No other differences in fatty acid composition were observed in the transgenic seeds.

Table 4
Composition of T2 Pooled Seed

| | 16:0 % | 16:1 % | 18:0 % | 18:1 % | (5,9)18:2 % | 18:2 % | (5,9,12)18:3 % | 18:3 % | 20:0 % | 20:1 % | 20:2 % | 22:0 % | 22:1 % | 24:0 % |
|---------------|-----------|-----------|-----------|-----------|----------------|-----------|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| LP004 control | 3.86 | 0.15 | 3.05 | 69.1 | 0 | 18.51 | 0.01 | 1.65 | 1.09 | 1.40 | 0.03 | 0.63 | 0.05 | 0.42 |
| 5531-1 | 4.26 | 0.15 | 3.23 | 62.33 | 4.07 | 21.44 | 0.33 | 1.38 | 0.91 | 1.04 | 0.05 | 0.41 | 0.03 | 0.27 |
| 5531-2 | 3.78 | 0.14 | 3.37 | 66.18 | 4.57 | 17.31 | 0.27 | 1.30 | 1.03 | 1.18 | 0 | 0.47 | 0.01 | 0.30 |
| 5531-6 | 3.78 | 0.13 | 3.47 | 63.61 | 6.21 | 17.97 | 0.38 | 1.34 | 1.04 | 1.14 | 0.05 | 0.49 | 0.02 | 0.26 |
| 5531-10 | 3.96 | 0.17 | 3.28 | 63.82 | 5.41 | 18.58 | 0.32 | 1.43 | 0.98 | 1.11 | 0.02 | 0.50 | 0 | 0.31 |
| 5531-16 | 3.91 | 0.17 | 3.33 | 64.31 | 5.03 | 18.98 | 0.33 | 1.39 | 0.96 | 1.11 | 0 | 0.44 | 0 | 0 |
| 5531-28 | 3.81 | 0.13 | 2.58 | 62.64 | 5.36 | 20.95 | 0.45 | 1.39 | 0.83 | 1.15 | 0.01 | 0.36 | 0.05 | 0.21 |
| | | | | | | | | | | | | | | |

Northern analysis is performed on plants to identify those expressing Ma29. Developing embryos are isolated approximately 25 days post anthesis or when the napin promoter is induced, and floated in a solution containing GLA or DGLA as described in Example 7. Fatty acid analysis of the embryos is then performed by GC to determine the amount of conversion of DGLA to ARA, following the protocol adapted for leaves in Example 7. The amount of ARA incorporated into triglycerides by endogenous *Brassica* acyltransferases is then evaluated by GC analysis as in Example 7.

Example 8

Expression of *M. alpina* $\Delta 6$ Desaturase in *Brassica napus*

The Ma524 cDNA was modified by PCR to introduce cloning sites using the following primers:

Ma524PCR-1 (SEQ ID NO:27)

5'-CUACUACUACUATCTAGACTCGAGACCATGGCTGCTGCT
CCAGTGTG

Ma524PCR-2 (SEQ ID NO:28)

5'-CAUCAUCAUCAUAGGCCTCGAGTTACTGCGCCTTACCCAT

These primers allowed the amplification of the entire coding region and added *Xba*I and *Xho*I sites to the 5'-end and *Xho*I and *Stu*I sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5535 and the $\Delta 6$ desaturase sequence was verified by sequencing of both strands.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5536. The *Not*I fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the *Not*I site of pCGN1557

to create pCGN5538. pCGN5538 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

5 Maturing T2 seeds were collected from 6 independent transformation events in the greenhouse. The fatty acid composition of single seeds was analyzed by GC. Table 5 shows the results of control LP004 seeds and six 5538 lines. All of the 5538 lines except #8 produced seeds containing GLA. Presence of GLA segregated in these seeds as is expected for the T2 selfed seed population. In addition to GLA, the *M. alpina* $\Delta 6$ desaturase is capable of producing 18:4 (stearidonic) and another fatty acid believed to be the 6,9-18:2.

10 The above results show that desaturases with three different substrate specificities can be expressed in a heterologous system and used to produce poly-unsaturated long chain fatty acids. Exemplified were the production of ARA (20:4) from the precursor 20:3 (DGLA), the production of GLA (18:3) from 18:2 substrate, and the conversion of 18:1 substrate to 18:2, which is the precursor for GLA.

15

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|-------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| LPO04-1 | 4.33 | 0.21 | 3.78 | 72.49 | 0 | 13.97 | 0 | 1.7 | 0 | 1.34 | 0.71 | 0.02 | 0.58 | 0.27 | |
| -2 | 4.01 | 0.16 | 3.09 | 73.59 | 0 | 14.36 | 0.01 | 1.4 | 0 | 1.43 | 0.66 | 0.02 | 0.5 | 0.2 | |
| -3 | 4.12 | 0.19 | 3.56 | 70.25 | 0 | 17.28 | 0 | 1.57 | 0 | 1.28 | 0.5 | 0.02 | 0.39 | 0.2 | |
| -4 | 4.22 | 0.2 | 2.7 | 70.25 | 0 | 17.86 | 0 | 1.61 | 0 | 1.31 | 0.53 | 0.02 | 0.4 | 0.24 | |
| -5 | 4.02 | 0.16 | 3.41 | 72.91 | 0 | 14.45 | 0.01 | 1.45 | 0 | 1.37 | 0.7 | 0.02 | 0.51 | 0.26 | |
| -6 | 4.22 | 0.18 | 3.23 | 71.47 | 0 | 15.92 | 0.01 | 1.52 | 0 | 1.32 | 0.69 | 0.02 | 0.51 | 0.27 | |
| -7 | 4.1 | 0.16 | 3.47 | 72.06 | 0 | 15.23 | 0 | 1.52 | 0 | 1.32 | 0.63 | 0.03 | 0.49 | 0.23 | |
| -9 | 4.01 | 0.17 | 3.71 | 72.98 | 0 | 13.97 | 0.01 | 1.41 | 0 | 1.45 | 0.74 | 0.03 | 0.58 | 0.23 | |
| -10 | 4.04 | 0.16 | 3.57 | 70.03 | 0 | 17.46 | 0 | 1.5 | 0 | 1.33 | 0.61 | 0.03 | 0.36 | 0.24 | |
| 5538-1-1 | 4.61 | 0.2 | 3.48 | 68.12 | 1.37 | 10.68 | 7.48 | 1.04 | 0.33 | 1.19 | 0.49 | 0.02 | 0.33 | 0.13 | |
| -2 | 4.61 | 0.22 | 3.46 | 68.84 | 1.36 | 10.28 | 7.04 | 1.01 | 0.31 | 1.15 | 0.48 | 0.02 | 0.39 | 0 | |
| -3 | 4.78 | 0.24 | 3.24 | 65.86 | 0 | 21.36 | 0 | 1.49 | 0 | 1.08 | 0.46 | 0.02 | 0.38 | 0.22 | |
| -4 | 4.84 | 0.3 | 3.89 | 67.64 | 1.67 | 9.9 | 6.97 | 1.02 | 0.36 | 1.14 | 0.53 | 0.02 | 0.5 | 0.18 | |
| -5 | 4.64 | 0.2 | 3.58 | 64.5 | 3.61 | 8.85 | 10.14 | 0.95 | 0.48 | 1.19 | 0.47 | 0.01 | 0.33 | 0.12 | |
| -6 | 4.91 | 0.27 | 3.44 | 66.51 | 1.48 | 11.14 | 7.74 | 1.15 | 0.33 | 1.08 | 0.49 | 0.02 | 0.34 | 0.13 | |
| -7 | 4.87 | 0.22 | 3.24 | 65.78 | 1.27 | 11.92 | 8.38 | 1.2 | 0 | 1.12 | 0.47 | 0.02 | 0.37 | 0.16 | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|------|------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -8 | 4.59 | 0.22 | 3.4 | 70.77 | 0 | 16.71 | 0 | 1.35 | 0 | 1.14 | 0.48 | 0.02 | 0.39 | 0.15 | | |
| -9 | 4.63 | 0.23 | 3.51 | 69.66 | 2.01 | 8.77 | 7.24 | 0.97 | 0 | 1.18 | 0.52 | 0.02 | 0.3 | 0.11 | | |
| -10 | 4.56 | 0.19 | 3.55 | 70.68 | 0 | 16.89 | 0 | 1.37 | 0 | 1.22 | 0.54 | 0.02 | 0.22 | 0.03 | | |
| 5538-3-1 | 4.74 | 0.21 | 3.43 | 67.52 | 1.29 | 10.91 | 7.77 | 1.03 | 0.28 | 1.11 | 0.5 | 0.02 | 0.35 | 0.14 | | |
| -2 | 4.72 | 0.21 | 3.24 | 67.42 | 1.63 | 10.37 | 8.4 | 0.99 | 0 | 1.12 | 0.49 | 0.02 | 0.36 | 0.15 | | |
| -3 | 4.24 | 0.21 | 3.52 | 71.31 | 0 | 16.53 | 0 | 1.33 | 0 | 1.12 | 0.45 | 0.02 | 0.4 | 0.14 | | |
| -4 | 4.64 | 0.21 | 3.45 | 67.92 | 1.65 | 9.91 | 7.97 | 0.91 | 0.33 | 1.14 | 0.47 | 0.02 | 0.37 | 0.14 | | |
| -5 | 4.91 | 0.25 | 3.31 | 67.19 | 0 | 19.92 | 0.01 | 1.39 | 0 | 1.05 | 0.48 | 0.02 | 0.37 | 0.14 | | |
| -6 | 4.67 | 0.21 | 3.25 | 67.07 | 1.23 | 11.32 | 8.35 | 0.99 | 0 | 1.16 | 0.47 | 0.02 | 0.33 | 0.16 | | |
| -7 | 4.53 | 0.19 | 2.94 | 64.8 | 4.94 | 8.45 | 9.95 | 0.93 | 0.44 | 1.13 | 0.37 | 0.01 | 0.27 | 0.12 | | |
| -8 | 4.66 | 0.22 | 3.68 | 67.33 | 0.71 | 12 | 6.99 | 1.1 | 0.24 | 1.18 | 0.48 | 0.03 | 0.36 | 0.17 | | |
| -9 | 4.65 | 0.24 | 3.11 | 67.42 | 0.64 | 12.71 | 6.93 | 1.16 | 0.25 | 1.08 | 0.45 | 0.02 | 0.32 | 0.17 | | |
| -10 | 4.88 | 0.27 | 3.33 | 65.75 | 0.86 | 12.89 | 7.7 | 1.1 | 0.24 | 1.08 | 0.46 | 0.01 | 0.34 | 0.16 | | |
| 5538-4-1 | 4.65 | 0.24 | 3.8 | 62.41 | 0 | 24.68 | 0 | 1.6 | 0.01 | 0.99 | 0.45 | 0.02 | 0.33 | 0.13 | | |
| -2 | 5.37 | 0.31 | 3 | 57.98 | 0.38 | 18.04 | 10.5 | 1.41 | 0 | 0.99 | 0.48 | 0.02 | 0.3 | 0.19 | | |
| -3 | 4.61 | 0.22 | 3.07 | 63.62 | 0.3 | 16.46 | 7.67 | 1.2 | 0 | 1.18 | 0.45 | 0.02 | 0.29 | 0.14 | | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|----------|------|------|------|-------|------|-------|-------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -4 | 4.39 | 0.19 | 2.93 | 65.97 | 0 | 22.36 | 0 | 1.45 | 0 | 1.17 | 0.41 | 0.03 | 0.32 | 0.15 | |
| -5 | 5.22 | 0.29 | 3.85 | 62.1 | 2.35 | 10.25 | 11.39 | 0.93 | 0.41 | 1.04 | 0.6 | 0.02 | 0.47 | 0.17 | |
| -6 | 4.66 | 0.18 | 2.85 | 66.79 | 0.5 | 13.03 | 7.66 | 0.97 | 0.22 | 1.28 | 0.42 | 0.02 | 0.31 | 0.14 | |
| -7 | 4.85 | 0.26 | 3.03 | 57.43 | 0.26 | 28.04 | 0.01 | 2.59 | 0.01 | 1.13 | 0.56 | 0.02 | 0.4 | 0.23 | |
| -8 | 5.43 | 0.28 | 2.94 | 54.8 | 1.84 | 13.79 | 15.67 | 1.36 | 0.53 | 1.1 | 0.55 | 0.02 | 0.35 | 0.19 | |
| -9 | 4.88 | 0.24 | 3.32 | 62.3 | 0.58 | 14.86 | 9.04 | 1.34 | 0.29 | 1.13 | 0.52 | 0.02 | 0.37 | 0.19 | |
| -10 | 4.53 | 0.2 | 2.73 | 64.2 | 0.07 | 24.15 | 0 | 1.52 | 0 | 1.09 | 0.39 | 0.02 | 0.27 | 0.17 | |
| 5538-5-1 | 4.5 | 0.15 | 3.35 | 66.71 | 0.88 | 11.7 | 8.38 | 1.04 | 0.3 | 1.24 | 0.49 | 0.02 | 0.29 | 0.17 | |
| -2 | 4.77 | 0.23 | 3.06 | 62.67 | 0.68 | 15.2 | 8.8 | 1.31 | 0.28 | 1.15 | 0.46 | 0.02 | 0.3 | 0.19 | |
| -3 | 4.59 | 0.22 | 3.61 | 64.35 | 2.29 | 9.95 | 10.57 | 1.01 | 0.45 | 1.21 | 0.48 | 0.02 | 0.26 | 0.16 | |
| -4 | 4.86 | 0.26 | 3.4 | 67.69 | 0.65 | 12.24 | 6.61 | 1.09 | 0.23 | 1.07 | 0.45 | 0.02 | 0.32 | 0.14 | |
| -5 | 4.49 | 0.21 | 3.3 | 69.25 | 0.04 | 16.51 | 2.18 | 1.2 | 0 | 1.11 | 0.44 | 0.02 | 0.33 | 0.16 | |
| -6 | 4.5 | 0.21 | 3.47 | 70.48 | 0.08 | 14.9 | 2.19 | 1.22 | 0 | 1.13 | 0.49 | 0.02 | 0.33 | 0.16 | |
| -7 | 4.39 | 0.21 | 3.44 | 67.59 | 2.38 | 9.24 | 8.98 | 0.89 | 0 | 1.18 | 0.44 | 0.02 | 0.28 | 0.14 | |
| -8 | 4.52 | 0.22 | 3.17 | 68.33 | 0.01 | 18.91 | 0.73 | 1.32 | 0.01 | 1.08 | 0.45 | 0.02 | 0.29 | 0.17 | |
| -9 | 4.68 | 0.2 | 3.05 | 64.03 | 1.93 | 11.03 | 11.41 | 1.02 | 0.01 | 1.15 | 0.39 | 0.02 | 0.21 | 0.15 | |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|-----------|------|------|------|-------|------|-------|------|--------|------|------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -10 | 4.57 | 0.2 | 3.1 | 67.21 | 0.61 | 12.62 | 7.68 | 1.07 | 0.25 | 1.14 | 0.43 | 0.02 | 0.02 | 0.25 | 0.15 |
| 5538-8-1 | 4.95 | 0.26 | 3.14 | 64.04 | 0 | 23.38 | 0 | 1.54 | 0 | 0.99 | 0.42 | 0.02 | 0.02 | 0.38 | 0.17 |
| -2 | 4.91 | 0.26 | 3.71 | 62.33 | 0 | 23.97 | 0 | 1.77 | 0 | 0.95 | 0.53 | 0.02 | 0.02 | 0.42 | 0.19 |
| -3 | 4.73 | 0.25 | 4.04 | 63.83 | 0 | 22.36 | 0.01 | 1.73 | 0 | 1.05 | 0.55 | 0.02 | 0.02 | 0.45 | 0.16 |
| -4 | 5.1 | 0.35 | 3.8 | 60.45 | 0 | 24.45 | 0.01 | 2.13 | 0 | 1.07 | 0.65 | 0.03 | 0.03 | 0.53 | 0.24 |
| -5 | 4.98 | 0.3 | 3.91 | 62.48 | 0 | 23.44 | 0 | 1.77 | 0 | 1.01 | 0.51 | 0.01 | 0.01 | 0.43 | 0.21 |
| -6 | 4.62 | 0.21 | 3.99 | 66.14 | 0 | 20.38 | 0 | 1.48 | 0 | 1.15 | 0.53 | 0.02 | 0.02 | 0.48 | 0.19 |
| -7 | 4.64 | 0.22 | 3.55 | 64.6 | 0 | 22.65 | 0 | 1.38 | 0 | 1.09 | 0.45 | 0.02 | 0.02 | 0.41 | 0.19 |
| -8 | 5.65 | 0.38 | 3.18 | 56.6 | 0 | 30.83 | 0.02 | 0.02 | 0 | 0.98 | 0.55 | 0.03 | 0.03 | 0.39 | 0.26 |
| -9 | 8.53 | 0.63 | 6.9 | 51.76 | 0 | 26.01 | 0 | 0.01 | 0 | 1.41 | 1.21 | 0.07 | 0.07 | 0.96 | 0.33 |
| -10 | 5.52 | 0.4 | 3.97 | 57.92 | 0 | 28.95 | 0 | 0.02 | 0 | 0.95 | 0.52 | 0.02 | 0.02 | 0.41 | 0.16 |
| 5538-10-1 | 4.44 | 0.19 | 3.5 | 68.42 | 0 | 19.51 | 0 | 1.32 | 0 | 1.14 | 0.45 | 0.02 | 0.02 | 0.31 | 0.16 |
| -2 | 4.57 | 0.21 | 3.07 | 66.08 | 0 | 21.99 | 0.01 | 1.36 | 0 | 1.12 | 0.41 | 0.02 | 0.02 | 0.31 | 0.16 |
| -3 | 4.63 | 0.21 | 3.48 | 67.43 | 0 | 20.27 | 0.01 | 1.32 | 0 | 1.12 | 0.46 | 0.02 | 0.02 | 0.21 | 0.08 |
| -4 | 4.69 | 0.19 | 3.22 | 64.62 | 0 | 23.16 | 0 | 1.35 | 0 | 1.08 | 0.46 | 0.02 | 0.02 | 0.33 | 0.2 |
| -5 | 4.58 | 0.2 | 3.4 | 68.75 | 0 | 20.17 | 0.01 | 0.02 | 0 | 1.1 | 0.45 | 0.02 | 0.02 | 0.34 | 0.17 |

Table 5
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

| SPL # | 16:0 | 16:1 | 18:0 | 18:1 | 6,9 | 18:2 | 18:2 | 18:3ga | 18:3 | 18:4 | 20:1 | 22:0 | 22:1 | 24:0 | 24:1 |
|-------|------|------|------|-------|------|-------|------|--------|------|------|------|------|------|------|------|
| % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| -8 | 4.55 | 0.21 | 0 | 73.55 | 0.05 | 14.91 | 2.76 | 1.21 | 0.07 | 1.24 | 0.51 | 0.02 | 0.19 | 0 | |
| -9 | 4.58 | 0.21 | 3.28 | 66.19 | 0 | 21.55 | 0 | 1.35 | 0 | 1.12 | 0.43 | 0.02 | 0.33 | 0.16 | |
| -10 | 4.52 | 0.2 | 3.4 | 68.37 | 0 | 19.33 | 0.01 | 1.3 | 0 | 1.13 | 0.46 | 0.02 | 0.35 | 0.18 | |

Example 9**Expression of *M. alpina* $\Delta 12$ desaturase in *Brassica napus***

The Ma648 cDNA was modified by PCR to introduce cloning sites using the following primers:

5 Ma648PCR-for (SEQ ID NO:29)
 5'-CUACUACUACUAGGATCCATGGCACCTCCCAACACT
 Ma648PCR-rev (SEQ ID NO:30)
 5'-CAUCAUCAUGGTACCTCGAGTTACTTCTTGAAAAAGAC

10 These primers allowed the amplification of the entire coding region and added a BamHI site to the 5' end and KpnI and XhoI sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5540 and the $\Delta 12$ desaturase sequence was verified by sequencing of both strands.

15 For seed-specific expression, the Ma648 coding region was cut out of pCGN5540 as a BamHI/XhoI fragment and inserted between the BglII and XhoI sites of the napin expression cassette, pCGN3223, to create pCGN5542. The Asp718 fragment of pCGN5541 containing the napin 5' regulatory region, the Ma648 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5542. PCGN5542 was
 20 introduced into two varieties of *Brassica napus* via *Agrobacterium* mediated transformation. The commercial canola variety, SP30021, and a low-linolenic line, LP30108 were used.

25 Mature selfed T2 seeds were collected from 19 independent LP30108 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 6. All transformed lines contained increased levels of 18:2, the product of the $\Delta 12$ desaturase. Levels of 18:3 were not significantly increased in these plants. Events # 11 and 16 showed the greatest accumulation

of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 7. Individual T2 seeds containing the *M. alpina* $\Delta 12$ desaturase accumulated up to 60% 18:2 in the seeds. Sample 97xx1116 #59 is an example of a null segregant. Even in the highest 18:2 accumulators, levels of 18:3 were increased only slightly. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed.

Mature selfed T2 seeds were collected from 20 independent SP30021 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The data are presented in Table 8. All transformed lines contained increased levels of 18:2, the product of the $\Delta 12$ desaturase. As in the low-linolenic LP30108 line, levels of 18:3 were not significantly increased. Events # 4 and 12 showed the greatest accumulation of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 9. Samples 97xx1157 #88 and #18 are examples of null segregants for 5542-SP30021-4 and 5542-SP30021-12 respectively. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed

Table 6

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| 97XX1098 | 45 | 5542-LP30108-16 | 7.04 | 0.43 | 1.12 | 18.01 | 66.36 | 4.76 | 0.5 | 0.84 | 0.3 | 0.44 |
| 97XX1098 | 22 | 5542-LP30108-16 | 5.17 | 0.29 | 2.11 | 22.01 | 65.18 | 3.15 | 0.63 | 0.75 | 0.21 | 0.36 |
| 97XX1098 | 40 | 5542-LP30108-16 | 4.99 | 0.2 | 2.05 | 23.91 | 63.13 | 3.3 | 0.73 | 0.85 | 0.23 | 0.49 |
| 97XX1098 | 28 | 5542-LP30108-16 | 4.47 | 0.19 | 1.75 | 26.7 | 62.39 | 2.46 | 0.58 | 0.85 | 0.2 | 0.32 |
| 97XX1098 | 2 | 5542-LP30108-16 | 4.54 | 0.21 | 1.66 | 26.83 | 61.89 | 2.9 | 0.55 | 0.82 | 0.18 | 0.33 |
| 97XX1098 | 58 | 5542-LP30108-16 | 6.05 | 0.31 | 1.36 | 24.11 | 61.36 | 3.8 | 0.72 | 1.13 | 0.26 | 0.58 |
| 97XX1098 | 83 | 5542-LP30108-16 | 5.13 | 0.17 | 2.03 | 27.05 | 60.93 | 2.62 | 0.7 | 0.71 | 0.14 | 0.4 |
| 97XX1098 | 34 | 5542-LP30108-16 | 4.12 | 0.19 | 1.44 | 29.35 | 60.54 | 2.53 | 0.43 | 0.89 | 0.17 | 0.25 |
| 97XX1116 | 37 | 5542-LP30108-11 | 4 | 0.14 | 2.43 | 23.29 | 63.99 | 2.6 | 0.58 | 0.69 | 0.71 | 1.11 |
| 97XX1116 | 88 | 5542-LP30108-11 | 3.8 | 0.18 | 2.04 | 23.59 | 63.93 | 2.95 | 0.54 | 0.81 | 0.99 | 0.82 |
| 97XX1116 | 36 | 5542-LP30108-11 | 4.15 | 0.2 | 1.51 | 25.94 | 62.14 | 2.74 | 0.47 | 0.87 | 0.79 | 0.81 |
| 97XX1116 | 31 | 5542-LP30108-11 | 6.29 | 0.35 | 1.04 | 24.14 | 60.91 | 4.02 | 0.55 | 0.91 | 0.75 | 0.72 |
| 97XX1116 | 10 | 5542-LP30108-11 | 6.97 | 0.4 | 3.36 | 18.9 | 60.66 | 4.68 | 1.2 | 0.7 | 0.53 | 1.71 |
| 97XX1116 | 32 | 5542-LP30108-11 | 3.96 | 0.16 | 2.61 | 26.73 | 60.54 | 3.38 | 0.66 | 0.87 | 0.2 | 0.62 |
| 97XX1116 | 55 | 5542-LP30108-11 | 4.26 | 0.22 | 0.98 | 28.57 | 59.94 | 3.24 | 0.4 | 0.68 | 0.71 | 0.75 |
| 97XX1116 | 12 | 5542-LP30108-11 | 4.17 | 0.23 | 1.42 | 28.61 | 59.52 | 3.26 | 0.51 | 0.95 | 0.29 | 0.67 |

Table 6

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| 97XX1116 | 86 | 5542-LP30108-11 | 4.23 | 0.3 | 1.09 | 28.34 | 59.2 | 3.95 | 0.48 | 0.91 | 0.55 | 0.71 |
| 97XX1116 | 61 | 5542-LP30108-11 | 4.13 | 0.16 | 1.92 | 30.18 | 58.67 | 2.65 | 0.56 | 0.88 | 0.25 | 0.41 |
| 97XX1116 | 60 | 5542-LP30108-11 | 4.42 | 0.26 | 1.61 | 28.77 | 58.6 | 3.26 | 0.53 | 0.85 | 0.68 | 0.75 |
| 97XX1116 | 91 | 5542-LP30108-11 | 7.82 | 0.67 | 2.37 | 17.97 | 58.43 | 4.85 | 0.94 | 0.86 | 3.87 | 1.71 |
| 97xx1116 | 59 | 5542-LP30108-11 | 3.56 | 0.2 | 1.6 | 65.5 | 23.03 | 2.23 | 0.52 | 1.54 | 0.49 | 0.69 |

Table 7

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % |
| 5542-LP30108-1 | 4.6 | 0.15 | 1.93 | 50.44 | 38.54 | 2.06 | 0.65 | 1.11 | 0.09 | 0.37 |
| 5542-LP30108-2 | 4.63 | 0.17 | 1.78 | 41.11 | 47.53 | 2.46 | 0.62 | 1.02 | 0.14 | 0.38 |
| 5542-LP30108-3 | 4.96 | 0.18 | 2.07 | 48.16 | 40.01 | 2.17 | 0.73 | 1.13 | 0.1 | 0.39 |
| 5542-LP30108-4 | 4.36 | 0.15 | 1.94 | 46.51 | 42.57 | 1.95 | 0.64 | 1.06 | 0.11 | 0.35 |
| 5542-LP30108-5 | 4.45 | 0.14 | 2.19 | 49.54 | 39.13 | 2.14 | 0.72 | 1.14 | 0.11 | 0.38 |
| 5542-LP30108-6 | 4.97 | 0.16 | 1.86 | 49.23 | 39.2 | 2.17 | 0.7 | 1.12 | 0.11 | 0.41 |
| 5542-LP30108-7 | 4.46 | 0.13 | 2.72 | 39.6 | 48.65 | 2.02 | 0.81 | 0.96 | 0.13 | 0.4 |
| 5542-LP30108-8 | 4.63 | 0.18 | 1.78 | 47.86 | 41 | 2.31 | 0.62 | 1.09 | 0.11 | 0.36 |
| 5542-LP30108-9 | 4.64 | 0.16 | 1.75 | 42.5 | 46.57 | 2.2 | 0.61 | 1 | 0.13 | 0.35 |
| 5542-LP30108-10 | 4.46 | 0.15 | 2.37 | 43.61 | 45.29 | 1.77 | 0.71 | 1.02 | 0.12 | 0.36 |
| 5542-LP30108-11 | 4.58 | 0.25 | 1.88 | 37.08 | 50.95 | 2.94 | 0.64 | 0.96 | 0.16 | 0.42 |
| 5542-LP30108-12 | 4.46 | 0.18 | 1.69 | 43.62 | 45.36 | 2.44 | 0.59 | 1.09 | 0.14 | 0.34 |
| 5542-LP30108-13 | 4.45 | 0.15 | 2.33 | 51 | 37.71 | 1.91 | 0.75 | 1.12 | 0.09 | 0.4 |
| 5542-LP30108-14 | 4.3 | 0.16 | 2.04 | 45.93 | 42.78 | 2.46 | 0.66 | 1.07 | 0.14 | 0.37 |
| 5542-LP30108-15 | 4.18 | 0.16 | 2.17 | 43.79 | 45.2 | 2.14 | 0.68 | 1.04 | 0.15 | 0.36 |
| 5542-LP30108-16 | 5.04 | 0.18 | 1.89 | 32.32 | 55.78 | 2.68 | 0.63 | 0.84 | 0.2 | 0.36 |

Table 7

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|------|------|------|------|------|
| | % | % | % | % | % | % | % | % | % | % |
| 5542-LP30108-18 | 4.2 | 0.14 | 2.23 | 50.63 | 38.51 | 1.79 | 0.72 | 1.15 | 0.1 | 0.37 |
| 5542-LP30108-19 | 4.63 | 0.18 | 1.81 | 52.51 | 36.26 | 2.12 | 0.68 | 1.19 | 0.1 | 0.4 |
| 5542-LP30108-20 | 4.77 | 0.15 | 2.78 | 39.76 | 48.06 | 2.25 | 0.75 | 0.91 | 0.13 | 0.36 |
| LP30108 control | 4.31 | 0.22 | 2.05 | 66.15 | 22.59 | 1.87 | 0.77 | 1.3 | 0.07 | 0.44 |

Table 8

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 5542-SP30021-1 | 4.37 | 0.17 | 2.17 | 40.26 | 39.43 | 11.06 | 0.74 | 1.14 | 0.14 | 0.42 |
| 5542-SP30021-2 | 4.33 | 0.18 | 1.51 | 43.07 | 36.03 | 12.57 | 0.57 | 1.21 | 0.14 | 0.33 |
| 5542-SP30021-3 | 5.2 | 0.22 | 3.1 | 43.7 | 37.04 | 8.03 | 0.92 | 1.06 | 0.13 | 0.48 |
| 5542-SP30021-4 | 4.37 | 0.15 | 1.94 | 34.26 | 45.12 | 12.04 | 0.6 | 0.96 | 0.17 | 0.3 |
| 5542-SP30021-5 | 4.15 | 0.17 | 1.73 | 48.98 | 31.13 | 11.41 | 0.63 | 1.26 | 0.13 | 0.35 |
| 5542-SP30021-6 | 4.52 | 0.17 | 1.92 | 38.1 | 42.39 | 10.53 | 0.67 | 1.04 | 0.18 | 0.39 |
| 5542-SP30021-7 | 4.58 | 0.18 | 1.66 | 41.87 | 37.52 | 11.8 | 0.62 | 1.14 | 0.15 | 0.36 |
| 5542-SP30021-8 | 4.46 | 0.17 | 1.59 | 42.69 | 36.93 | 11.88 | 0.59 | 1.14 | 0.14 | 0.35 |
| 5542-SP30021-9 | 4.63 | 0.19 | 1.69 | 39.89 | 39.75 | 11.48 | 0.62 | 1.09 | 0.15 | 0.38 |
| 5542-SP30021-10 | 4.74 | 0.16 | 1.79 | 39.19 | 40.51 | 11.42 | 0.63 | 0.99 | 0.13 | 0.34 |
| 5542-SP30021-11 | 4.57 | 0.16 | 1.71 | 38.13 | 42 | 11.15 | 0.62 | 1.04 | 0.18 | 0.36 |
| 5542-SP30021-12 | 4.05 | 0.16 | 2.04 | 35.44 | 43.47 | 12.45 | 0.62 | 1.07 | 0.21 | 0.33 |
| 5542-SP30021-13 | 4.37 | 0.15 | 1.79 | 38.74 | 41.28 | 11.36 | 0.62 | 1.04 | 0.16 | 0.35 |
| 5542-SP30021-14 | 4.32 | 0.16 | 1.47 | 42.32 | 37.17 | 12.3 | 0.54 | 1.16 | 0.16 | 0.32 |
| 5542-SP30021-15 | 4.25 | 0.18 | 1.65 | 44.96 | 34.28 | 12.39 | 0.59 | 1.13 | 0.14 | 0.32 |

Table 8

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 5542-SP30021-16 | 4.53 | 0.17 | 1.91 | 42.13 | 38.32 | 10.51 | 0.67 | 1.12 | 0.14 | 0.38 |
| 5542-SP30021-17 | 4.16 | 0.19 | 1.7 | 50.65 | 29.3 | 11.4 | 0.61 | 1.29 | 0.11 | 0.36 |
| 5542-SP30021-18 | 4.24 | 0.17 | 1.68 | 44.47 | 35.46 | 11.52 | 0.6 | 1.19 | 0.14 | 0.34 |
| 5542-SP30021-19 | 4.1 | 0.18 | 1.8 | 46.67 | 33.87 | 10.86 | 0.63 | 1.24 | 0.13 | 0.37 |
| 5542-SP30021-20 | 4.3 | 0.17 | 1.64 | 39.6 | 40.39 | 11.53 | 0.57 | 1.12 | 0.16 | 0.32 |
| SP30021 | 4.38 | 0.21 | 1.47 | 56.51 | 22.59 | 12.04 | 0.62 | 1.45 | 0.11 | 0.39 |

Table 9

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 97XX1156 | 96 | 5542-SP30021-4 | 3.71 | 0.13 | 1.36 | 29.29 | 51.74 | 11.57 | 0.41 | 0.85 | 0.18 | 0.46 |
| 97XX1156 | 50 | 5542-SP30021-4 | 2.95 | 0.11 | 1.33 | 28.78 | 50.97 | 13.83 | 0.3 | 0.99 | 0.28 | 0.32 |
| 97XX1158 | 10 | 5542-SP30021-4 | 4.05 | 0.16 | 2.47 | 31.18 | 50.88 | 8.77 | 0.67 | 0.89 | 0.22 | 0.33 |
| 97XX1158 | 32 | 5542-SP30021-4 | 3.56 | 0.15 | 1.44 | 30.73 | 50.1 | 11.86 | 0.47 | 0.91 | 0.21 | 0.22 |
| 97XX1158 | 56 | 5542-SP30021-4 | 4.44 | 0.19 | 3.09 | 30.64 | 49.71 | 9.39 | 0.83 | 0.79 | 0.2 | 0.4 |
| 97XX1157 | 80 | 5542-SP30021-4 | 4.05 | 0.18 | 1.32 | 27.41 | 49.59 | 14.81 | 0.53 | 1.19 | 0.29 | 0.4 |
| 97XX1158 | 39 | 5542-SP30021-4 | 4.04 | 0.15 | 2.98 | 28.62 | 49.52 | 12.28 | 0.69 | 0.86 | 0.31 | 0.27 |
| 97XX1156 | 17 | 5542-SP30021-4 | 3.65 | 0.15 | 2.43 | 29.38 | 49.42 | 12.3 | 0.52 | 0.92 | 0.67 | 0.35 |
| 97XX1156 | 60 | 5542-SP30021-4 | 3.75 | 0.17 | 1.7 | 30.03 | 49.13 | 12.87 | 0.51 | 1.01 | 0.27 | 0.35 |
| 97XX1157 | 83 | 5542-SP30021-4 | 4.15 | 0.2 | 1.77 | 29.72 | 49.08 | 12.22 | 0.66 | 1.21 | 0.16 | 0.52 |
| 97XX1157 | 86 | 5542-SP30021-4 | 3.6 | 0.14 | 1.12 | 27.65 | 49.01 | 16.05 | 0.48 | 1.21 | 0.33 | 0.08 |
| 97XX1158 | 77 | 5542-SP30021-4 | 4.14 | 0.17 | 1.58 | 31.98 | 48.82 | 10.72 | 0.65 | 1 | 0.28 | 0.44 |
| 97XX1157 | 88 | 5542-SP30021-4 | 3.36 | 0.15 | 1.22 | 56.42 | 21.63 | 13.78 | 0.58 | 1.85 | 0.06 | 0.65 |

Table 9

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:0 | 20:1 | 20:2 | 22:0 |
|----------|--------|-----------------|------|------|------|-------|-------|-------|------|------|------|------|
| 97XX1157 | 39 | 5542-SP30021-12 | 2.84 | 0.04 | 1.84 | 29.6 | 53.16 | 9.52 | 0.57 | 1.32 | 0.35 | 0.48 |
| 97XX1157 | 55 | 5542-SP30021-12 | 3.28 | 0.1 | 2.18 | 30.36 | 52.27 | 9.26 | 0.63 | 1.15 | 0.22 | 0.41 |
| 97XX1157 | 10 | 5542-SP30021-12 | 3.5 | 0.06 | 1.51 | 29.78 | 50.98 | 11.13 | 0.64 | 1.45 | 0.4 | 0.26 |
| 97XX1157 | 41 | 5542-SP30021-12 | 3.31 | 0.08 | 1.64 | 30.18 | 50.51 | 11.59 | 0.57 | 1.27 | 0.24 | 0.41 |
| 97XX1157 | 35 | 5542-SP30021-12 | 3.31 | 0.09 | 1.57 | 30.36 | 50.1 | 12.17 | 0.5 | 1.15 | 0.23 | 0.35 |
| 97XX1157 | 1 | 5542-SP30021-12 | 3.45 | 0.11 | 2.88 | 32.11 | 49.45 | 8.69 | 0.82 | 1.22 | 0.27 | 0.63 |
| 97XX1157 | 16 | 5542-SP30021-12 | 2.91 | 0.09 | 1.52 | 29.35 | 48.88 | 14.26 | 0.58 | 1.39 | 0.15 | 0.3 |
| 97XX1157 | 50 | 5542-SP30021-12 | 3.29 | 0.09 | 2.13 | 33.23 | 48.78 | 9.87 | 0.67 | 1.06 | 0.18 | 0.47 |
| 97XX1157 | 25 | 5542-SP30021-12 | 2.83 | 0.05 | 1.4 | 33.22 | 48.52 | 11.22 | 0.5 | 1.33 | 0.26 | 0.42 |
| 97XX1157 | 57 | 5542-SP30021-12 | 2.94 | 0.13 | 1.46 | 32.85 | 47.58 | 12.21 | 0.57 | 1.31 | 0.27 | 0.47 |
| 97XX1157 | 56 | 5542-SP30021-12 | 3.01 | 0.07 | 1.63 | 31.53 | 47 | 14.02 | 0.59 | 1.31 | 0.28 | 0.23 |
| 97XX1157 | 6 | 5542-SP30021-12 | 3.9 | 0.13 | 1.5 | 32.43 | 46.98 | 12.45 | 0.52 | 1.11 | 0.21 | 0.49 |
| 97XX1157 | 18 | 5542-SP30021-12 | 3.88 | 0.16 | 1.73 | 57.94 | 22.33 | 10.51 | 0.74 | 1.68 | 0.11 | 0.64 |

Example 10

Simultaneous expression of *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5 In order to express the *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases from the same T-DNA, the following construct for seed-specific expression was made.

The NotI fragment of pCGN5536 containing the containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5542 to create pCGN5544. The
10 expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma648 and the nptII marker is the same.

PCGN5544 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 16 independent LP30108 transformation events and a non-transformed
15 control that were grown in the greenhouse. These seeds are expected to be segregating for the $\Delta 6 + \Delta 12$ desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are presented in Table 10. All but one of the lines (5544-LP30108-3) shows an altered oil composition as compared to the controls. GLA was produced in all but three of the lines (-3, -4,
20 -11); two of the three without GLA (-4, -11) showed increased 18:2 indicative of expression of the $\Delta 12$ desaturase. As a group, the levels of GLA observed in plants containing the double $\Delta 6 + \Delta 12$ construct (pCGN5544) were higher than those of plants containing pCGN5538 ($\Delta 6$ alone). In addition, levels of the $\Delta^{6,9}$ 18:2 are much reduced in the plants containing the $\Delta 12 + \Delta 6$ as compared to $\Delta 6$
25 alone. Thus, the combination of $\Delta 6$ and $\Delta 12$ desaturases on one T-DNA leads to the accumulation of more GLA and fewer side products than expression of $\Delta 6$ desaturase alone. To investigate the segregation of GLA levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30
30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of

these analyses are shown in Table 11. As expected for the T2 population, levels of GLA and 18:2 are segregating in the individual seeds. GLA content of up to 60% of total fatty acids was observed in individual seeds. Individual events were selected to be grown in the greenhouse and field for production of T3 seed.

Transgenic plants including *Brassica*, soybean, safflower, corn flax and sunflower expressing the constructs of this invention can be a good source of GLA.

Typical sources of GLA such as borage produce at most 25% GLA. In contrast the plants in Table 10 contain up to 30% GLA. Furthermore, the individual seeds shown in Table 11 contain up to 60% GLA.

Table 10

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:2 | 18:2 | 18:3 | 18:3 | 18:4 | 20:0 | 20:1 | 22:0 |
|-----------------|------|------|------|-------|------|--------------|---------------|-----------------|------------------|------|------|------|------|
| | | | | | | $\Delta 6,9$ | $\Delta 9,12$ | $\Delta 6,9,12$ | $\Delta 9,12,15$ | | | | |
| | % | % | % | % | % | % | % | % | % | % | % | % | % |
| 5544-LP30108-1 | 4.54 | 0.17 | 1.91 | 49.96 | 0 | 30.98 | 7.97 | 1.85 | 0.11 | 0.68 | 1.17 | 0.41 | |
| 5544-LP30108-2 | 4.69 | 0.19 | 2.15 | 38.49 | 0 | 33.94 | 16.21 | 1.73 | 0.25 | 0.72 | 0.96 | 0.41 | |
| 5544-LP30108-3 | 4.26 | 0.2 | 1.97 | 66.68 | 0 | 22.13 | 0.08 | 1.96 | 0.01 | 0.73 | 1.33 | 0.42 | |
| 5544-LP30108-4 | 4.59 | 0.24 | 1.76 | 44.21 | 0 | 44.54 | 0.02 | 2.19 | 0.01 | 0.62 | 1.08 | 0.4 | |
| 5544-LP30108-5 | 4.5 | 0.18 | 2.28 | 47.57 | 0 | 26.41 | 14.42 | 1.71 | 0.22 | 0.78 | 1.1 | 0.43 | |
| 5544-LP30108-6 | 4.51 | 0.16 | 2.12 | 31.95 | 0.01 | 26.94 | 29.8 | 1.41 | 0.5 | 0.81 | 1.02 | 0.51 | |
| 5544-LP30108-7 | 4.84 | 0.21 | 1.68 | 38.24 | 0 | 32.27 | 18.21 | 1.87 | 0.33 | 0.66 | 1.04 | 0.43 | |
| 5544-LP30108-10 | 5 | 0.28 | 1.86 | 41.17 | 0 | 46.54 | 0.36 | 2.58 | 0.02 | 0.6 | 0.91 | 0.37 | |
| 5544-LP30108-11 | 4.57 | 0.2 | 1.74 | 47.29 | 0 | 41.49 | 0.03 | 2.22 | 0.01 | 0.64 | 1.17 | 0.4 | |
| 5544-LP30108-12 | 4.87 | 0.18 | 2.65 | 34.53 | 0 | 30.37 | 23.12 | 1.46 | 0.36 | 0.83 | 0.95 | 0.45 | |
| 5544-LP30108-13 | 4.41 | 0.16 | 2.32 | 40.82 | 0.11 | 26.8 | 21.05 | 1.53 | 0.37 | 0.77 | 1.06 | 0.42 | |
| 5544-LP30108-14 | 4.38 | 0.2 | 2.21 | 29.91 | 0.16 | 28.01 | 30.62 | 1.46 | 0.59 | 0.76 | 0.97 | 0.47 | |
| 5544-LP30108-15 | 4.79 | 0.22 | 2.23 | 23.42 | 0.02 | 28.73 | 35.68 | 1.51 | 0.77 | 0.87 | 0.89 | 0.56 | |
| 5544-LP30108-16 | 4.54 | 0.18 | 1.78 | 40.81 | 0 | 35.24 | 12.83 | 1.95 | 0.27 | 0.68 | 1.02 | 0.43 | |
| 5544-LP30108-17 | 4.63 | 0.18 | 2.28 | 46.96 | 0 | 31.06 | 10.6 | 1.7 | 0.14 | 0.76 | 1.06 | 0.42 | |
| 5544-LP30108-20 | 4.87 | 0.29 | 1.44 | 31.81 | 0.15 | 23.51 | 32.85 | 1.64 | 0.69 | 0.89 | 0.96 | 0.67 | |

Table 10

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:2 | 18:3 | 18:3 | 18:4 | 20:0 | 20:1 | 22:0 |
|-----------------|------|------|------|-------|--------------|---------------|-----------------|------------------|------|------|------|------|
| | | | | | $\Delta 6,9$ | $\Delta 9,12$ | $\Delta 6,9,12$ | $\Delta 9,12,15$ | | | | |
| | % | % | % | % | % | % | % | % | % | % | % | % |
| LP30108 control | 3.89 | 0.25 | 1.19 | 67.73 | 0 | 22.46 | 0.1 | 1.97 | 0 | 0.54 | 1.32 | 0.44 |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1333 | 64 | 5544-LP30108-20 | 6.53 | 0.15 | 0.98 | 23.33 | 0.01 | 21.1 | 43.3 | 1.34 | 0.84 | 0.52 | 0.97 |
| 97XX1333 | 65 | 5544-LP30108-20 | 6.9 | 0.29 | 1.17 | 8.89 | 0.03 | 15.07 | 60.5 | 1.12 | 2.23 | 0.98 | 0.86 |
| 97XX1333 | 66 | 5544-LP30108-20 | 8.15 | 0.2 | 3.6 | 16.87 | 0.11 | 16.05 | 48.23 | 1.1 | 1.18 | 1.71 | 0.66 |
| 97XX1333 | 67 | 5544-LP30108-20 | 8.85 | 0.35 | 1.2 | 14.49 | 0.01 | 25.66 | 43.98 | 1.8 | 1.03 | 0.65 | 0.76 |
| 97XX1333 | 68 | 5544-LP30108-20 | 6.05 | 0.16 | 1.27 | 17.85 | 0.16 | 16.13 | 53.16 | 1.14 | 1.25 | 0.71 | 0.85 |
| 97XX1333 | 69 | 5544-LP30108-20 | 7.16 | 0.21 | 1.33 | 11.51 | 0.09 | 17.42 | 56.13 | 1.41 | 1.58 | 0.93 | 0.68 |
| 97XX1333 | 70 | 5544-LP30108-20 | 3.46 | 0.04 | 1.76 | 18.38 | 0.03 | 22.55 | 48.55 | 1.22 | 1.04 | 0.83 | 0.95 |
| 97XX1333 | 71 | 5544-LP30108-20 | 3.71 | 0.05 | 1.74 | 16.11 | 0.01 | 26.93 | 45.79 | 1.47 | 1.02 | 0.89 | 1 |
| 97XX1333 | 72 | 5544-LP30108-20 | 3.5 | 0.04 | 1.76 | 23.74 | 0.02 | 35.38 | 30.82 | 1.87 | 0.58 | 0.65 | 0.89 |
| 97XX1333 | 73 | 5544-LP30108-20 | 4.67 | 0.11 | 1.87 | 17.98 | 0.04 | 22.47 | 47.89 | 1.17 | 0.89 | 0.93 | 0.88 |
| 97XX1333 | 74 | 5544-LP30108-20 | 4.52 | 0.09 | 1.86 | 13.77 | 0.03 | 20.9 | 52.96 | 1.31 | 1.19 | 1.03 | 0.88 |
| 97XX1333 | 75 | 5544-LP30108-20 | 5.26 | 0.13 | 1.64 | 16.46 | 0.05 | 21.75 | 49.42 | 1.25 | 1.08 | 0.83 | 0.86 |
| 97XX1333 | 76 | 5544-LP30108-20 | 7.61 | 0.21 | 1.44 | 12.49 | 0.33 | 17 | 55.31 | 1.18 | 1.59 | 0.88 | 0.74 |
| 97XX1333 | 77 | 5544-LP30108-20 | 6.42 | 0.15 | 1.51 | 10.79 | 0.09 | 15.96 | 58.77 | 1.12 | 1.53 | 0.98 | 0.85 |
| 97XX1333 | 78 | 5544-LP30108-20 | 4.59 | 0.16 | 0.93 | 12.1 | 0.08 | 15.94 | 60.15 | 1.12 | 1.69 | 0.74 | 0.88 |
| 97XX1333 | 79 | 5544-LP30108-20 | 5.24 | 0.09 | 1.94 | 14.08 | 0.21 | 19.79 | 53.58 | 1.05 | 1.03 | 0.96 | 0.84 |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1333 | 80 | 5544-LP30108-20 | 4.38 | 0.08 | 1.66 | 22.25 | 0 | 30.79 | 35.49 | 2.16 | 0.72 | 0.66 | 0.84 |
| 97XX1333 | 81 | 5544-LP30108-20 | 4.05 | 0.05 | 1.44 | 24.16 | 0.04 | 24.86 | 40.89 | 1.42 | 0.79 | 0.63 | 0.84 |
| 97XX1333 | 82 | 5544-LP30108-20 | 3.29 | 0.05 | 1.9 | 19.66 | 0 | 23.83 | 46.48 | 1.27 | 0.87 | 0.78 | 0.81 |
| 97XX1333 | 83 | 5544-LP30108-20 | 4.82 | 0.08 | 1.99 | 17.27 | 0.1 | 20.69 | 49.73 | 1.22 | 1.06 | 0.98 | 0.82 |
| 97XX1333 | 84 | 5544-LP30108-20 | 5.33 | 0.1 | 1.77 | 13.6 | 0.03 | 21.44 | 51.74 | 1.52 | 1.21 | 0.98 | 0.93 |
| 97XX1333 | 85 | 5544-LP30108-20 | 3.3 | 0.05 | 1.2 | 68.23 | 0 | 22.09 | 0.01 | 2.27 | 0 | 0.57 | 1.57 |
| 97XX1333 | 86 | 5544-LP30108-20 | 3.23 | 0.05 | 1.54 | 28.15 | 0.01 | 36.4 | 25.91 | 1.99 | 0.43 | 0.59 | 0.97 |
| 97XX1333 | 87 | 5544-LP30108-20 | 4.38 | 0.1 | 1.16 | 60.94 | 2.85 | 8.35 | 17.61 | 1.26 | 0.69 | 0.54 | 1.39 |
| 97XX1333 | 88 | 5544-LP30108-20 | 4.4 | 0.09 | 1.34 | 38.42 | 0.02 | 34.74 | 16.61 | 2.12 | 0.32 | 0.53 | 0.82 |
| 97XX1278 | 16 | 5544-LP30108-15 | 3.62 | 0.11 | 1.22 | 27.23 | 0 | 30.9 | 32.87 | 1.41 | 0.48 | 0.46 | 0.97 |
| 97XX1278 | 17 | 5544-LP30108-15 | 3.68 | 0.13 | 1.26 | 45.29 | 0 | 44.79 | 0.72 | 1.77 | 0.01 | 0.43 | 1.24 |
| 97XX1278 | 18 | 5544-LP30108-15 | 4.08 | 0.15 | 1.49 | 22.34 | 0 | 28.37 | 39.37 | 1.22 | 0.64 | 0.55 | 0.88 |
| 97XX1278 | 19 | 5544-LP30108-15 | 3.51 | 0.1 | 1.01 | 35.44 | 0 | 44.12 | 11.7 | 1.72 | 0.15 | 0.36 | 1.14 |
| 97XX1278 | 20 | 5544-LP30108-15 | 3.66 | 0.12 | 1.21 | 27.44 | 0 | 30.2 | 32.37 | 1.49 | 0.53 | 0.49 | 1.15 |
| 97XX1278 | 21 | 5544-LP30108-15 | 3.58 | 0.11 | 1.51 | 29.81 | 0 | 30.72 | 30.65 | 1.16 | 0.4 | 0.5 | 0.96 |
| 97XX1278 | 23 | 5544-LP30108-15 | 3.69 | 0.11 | 1.42 | 30.05 | 0 | 32.28 | 27.41 | 1.65 | 0.38 | 0.54 | 1.19 |
| 97XX1278 | 24 | 5544-LP30108-15 | 3.56 | 0.11 | 1.31 | 30.25 | 0 | 28.64 | 31.46 | 1.43 | 0.48 | 0.48 | 1.11 |

Table 11

| CYCLE ID | SPL NO | STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|----------|--------|-----------------|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| 97XX1278 | 25 | 5544-LP30108-15 | 4.41 | 0.22 | 2.08 | 15.05 | 0 | 23.77 | 49.51 | 1.18 | 0.96 | 0.87 | 0.85 |
| 97XX1278 | 26 | 5544-LP30108-15 | 3.75 | 0.14 | 1.59 | 23.55 | 0 | 27.91 | 38.8 | 1.39 | 0.61 | 0.59 | 0.97 |
| 97XX1278 | 27 | 5544-LP30108-15 | 3.67 | 0.11 | 1.9 | 26.07 | 0 | 31.1 | 33.16 | 1.08 | 0.49 | 0.65 | 0.97 |
| 97XX1278 | 28 | 5544-LP30108-15 | 3.82 | 0.11 | 1.54 | 21.27 | 0 | 29.07 | 39.69 | 1.47 | 0.7 | 0.58 | 0.86 |
| 97XX1278 | 29 | 5544-LP30108-15 | 3.65 | 0.14 | 1.27 | 45.84 | 0 | 43.38 | 1 | 2.33 | 0.02 | 0.42 | 1.27 |
| 97XX1278 | 30 | 5544-LP30108-15 | 3.59 | 0.12 | 1.19 | 30.41 | 0 | 30.68 | 30.37 | 1.24 | 0.4 | 0.37 | 0.99 |
| 97XX1278 | 31 | 5544-LP30108-15 | 3.74 | 0.12 | 1.26 | 38.98 | 0 | 50.53 | 0.98 | 2.12 | 0.02 | 0.39 | 1.14 |
| 97XX1278 | 32 | 5544-LP30108-15 | 3.86 | 0.11 | 1.46 | 26.38 | 0 | 28.9 | 35.41 | 1.01 | 0.5 | 0.54 | 0.97 |

Example 11

Simultaneous expression of *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases in *Brassica napus*

5 In order to produce arachadonic acid (ARA) in transgenic canola oil both $\Delta 5$ and $\Delta 6$ desaturase activities need to be introduced. In order to facilitate downstream characterization and breeding, it may be advantageous to have both activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of $\Delta 5$ and $\Delta 6$ desaturases.

10 The Asp718 fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5545. The NotI fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5545
15 to create pCGN5546. The expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma29 and the nptII marker is the same.

 PCGN5546 was introduced into *Brassica napus* cv.LP30108 via
20 *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 12. All the lines show expression of both desaturases as evidenced by the presence of $\Delta^{5,9}$ 18:2 (as seen in pCGN5531 plants) and $\Delta^{6,9}$ 18:2 and GLA (as seen in pCGN5538 plants)

25

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5,9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|-----------------|------|------|------|-------|-----------|-----------|------------|------------------|-------------------|------|------|------|
| 5546-LP30108-1 | 4.88 | 0.33 | 2.28 | 57.2 | 4.68 | 6.08 | 7.36 | 12.29 | 1.38 | 0.85 | 0.84 | 1.22 |
| 5546-LP30108-2 | 4.01 | 0.14 | 2.22 | 66.04 | 2.73 | 1.33 | 12.6 | 6.45 | 1.41 | 0.32 | 0.75 | 1.2 |
| 5546-LP30108-3 | 4.29 | 0.15 | 2.55 | 68.89 | 0.44 | 0.58 | 16.97 | 1.66 | 1.6 | 0.11 | 0.88 | 1.22 |
| 5546-LP30108-4 | 4.24 | 0.14 | 2.6 | 70.48 | 0.73 | 0.52 | 14.28 | 2.61 | 1.42 | 0.14 | 0.96 | 1.26 |
| 5546-LP30108-5 | 3.52 | 0.15 | 2.01 | 60.3 | 1.72 | 0.95 | 16.92 | 9.88 | 1.66 | 0.39 | 0.68 | 1.26 |
| 5546-LP30108-6 | 4.05 | 0.17 | 2.24 | 61.29 | 1.98 | 0.4 | 18.87 | 6.28 | 2 | 0.34 | 0.7 | 1.24 |
| 5546-LP30108-7 | 4.74 | 0.21 | 2.49 | 64.5 | 2.25 | 1.18 | 10.03 | 9.73 | 1.35 | 0.52 | 0.97 | 1.28 |
| 5546-LP30108-8 | 4.24 | 0.14 | 2.82 | 63.92 | 1.9 | 1.5 | 11.67 | 9.29 | 1.44 | 0.43 | 0.89 | 1.19 |
| 5546-LP30108-9 | 3.8 | 0.13 | 2.15 | 65.75 | 2.3 | 0.16 | 14.92 | 6.32 | 1.57 | 0.24 | 0.75 | 1.35 |
| 5546-LP30108-10 | 4.28 | 0.17 | 1.55 | 58.8 | 1.1 | 0.12 | 22.95 | 5.97 | 2.24 | 0.22 | 0.6 | 1.35 |
| 5546-LP30108-11 | 4.25 | 0.15 | 1.82 | 63.68 | 1.01 | 0.22 | 19.42 | 4.96 | 1.81 | 0.2 | 0.67 | 1.23 |
| 5546-LP30108-12 | 3.95 | 0.14 | 2.36 | 66.9 | 1.12 | 0.01 | 19.42 | 1.59 | 1.77 | 0.04 | 0.8 | 1.21 |
| 5546-LP30108-13 | 4.18 | 0.16 | 2.17 | 66.91 | 1.36 | 0.02 | 18.84 | 1.99 | 1.74 | 0.05 | 0.77 | 1.15 |
| 5546-LP30108-14 | 4.74 | 0.26 | 1.82 | 65.29 | 1.25 | 0.27 | 16.77 | 5.3 | 1.59 | 0.25 | 0.71 | 1.32 |
| 5546-LP30108-15 | 4.3 | 0.23 | 2.54 | 65.65 | 1.67 | 0.59 | 13.15 | 7.22 | 1.54 | 0.36 | 0.88 | 1.3 |
| 5546-LP30108-16 | 4.05 | 0.17 | 2.75 | 64.13 | 2.56 | 2.8 | 9.56 | 9.31 | 1.34 | 0.53 | 0.92 | 1.28 |

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5,9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 |
|-----------------|------|------|------|-------|-----------|-----------|------------|------------------|-------------------|------|------|------|
| 5546-LP30108-17 | 4.06 | 0.13 | 2.85 | 65.76 | 2.09 | 1.92 | 9.65 | 9.1 | 1.23 | 0.45 | 0.92 | 1.22 |
| 5546-LP30108-18 | 4.16 | 0.25 | 2.14 | 60.68 | 1.43 | 0.02 | 24.02 | 2.62 | 2.11 | 0.09 | 0.69 | 1.26 |
| 5546-LP30108-19 | 5.77 | 0.37 | 2.15 | 56.11 | 1.6 | 0.33 | 19.34 | 9.16 | 2.37 | 0.46 | 0.73 | 1.05 |
| 5546-LP30108-20 | 5.03 | 0.36 | 2.34 | 61.05 | 1.55 | 0.35 | 17.21 | 6.96 | 2.24 | 0.39 | 0.77 | 1.22 |
| 5546-LP30108-21 | 4.52 | 0.3 | 2.71 | 62.14 | 1.33 | 0.23 | 17.62 | 6.44 | 1.88 | 0.28 | 0.88 | 1.15 |
| 5546-LP30108-22 | 5.91 | 0.44 | 2.15 | 60.12 | 1.41 | 0.36 | 17.04 | 7.75 | 1.97 | 0.36 | 0.78 | 1.07 |
| 5546-LP30108-23 | 4.28 | 0.22 | 2.44 | 66.19 | 0.93 | 0.11 | 17.03 | 4.37 | 1.67 | 0.17 | 0.82 | 1.25 |
| 5546-LP30108-24 | 4.92 | 0.33 | 2.68 | 62.6 | 1.32 | 0.36 | 16.89 | 5.82 | 2.05 | 0.3 | 0.95 | 1.19 |
| 5546-LP30108-25 | 5.42 | 0.72 | 3.15 | 47.47 | 2.66 | 4.21 | 13.51 | 16.31 | 2.14 | 0.99 | 1.18 | 1.37 |
| 5546-LP30108-26 | 3.85 | 0.22 | 2.78 | 65.02 | 1.05 | 0.05 | 18.35 | 4.36 | 1.67 | 0.12 | 0.82 | 1.18 |
| 5546-LP30108-27 | 3.86 | 0.15 | 2.76 | 65.17 | 1.11 | 0.78 | 16.24 | 5.21 | 1.53 | 0.25 | 0.93 | 1.3 |
| 5546-LP30108-28 | 5.29 | 0.42 | 1.81 | 49.12 | 1.07 | 0.09 | 30.52 | 5.21 | 3.57 | 0.44 | 0.67 | 1.23 |
| 5546-LP30108-29 | 4.4 | 0.2 | 2.38 | 65.95 | 1.05 | 0.28 | 16.31 | 4.85 | 1.64 | 0.19 | 0.85 | 1.26 |
| 5546-LP30108-30 | 3.99 | 0.19 | 2.55 | 67.47 | 0.83 | 0.11 | 17.02 | 3.18 | 1.68 | 0.13 | 0.83 | 1.23 |

Example 12

Simultaneous expression of *M. alpina* $\Delta 5$, $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5 In order to achieve optimal production of ARA in transgenic canola oil both the $\Delta 6$ and $\Delta 12$ desaturase activities may need to be present in addition to the $\Delta 5$ activity. In order to facilitate downstream characterization and breeding, it may be advantageous to have all of these activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of $\Delta 5$, $\Delta 6$
10 and $\Delta 12$ desaturases.

 The HindIII fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the HindIII site of pCGN5544 to create pCGN5547. The expression modules were oriented in such a way that the direction of transcription from
15 Ma29, Ma524, Ma648 and the nptII marker is the same.

 PCGN5547 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC.
20 The results are shown in Table 13. Twenty-seven of the lines show significant accumulation of GLA and in general the levels of GLA observed are higher than those seen in the 5546 plants that did not contain the $\Delta 12$ desaturase. The $\Delta 12$ desaturase appears to be active in most lines as evidenced by the lack of detectable $\Delta 6,9$ 18:2 and elevated 18:2 levels in most plants. Small amounts of
25 $\Delta 5,9$ 18:2 are seen in the 5547 plants, although the levels are generally less than those observed in the 5546 plants. This may be due to the presence of the $\Delta 12$ desaturase which efficiently converts the 18:1 to 18:2 before it can be desaturated at the $\Delta 5$ position.

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

| STRAIN ID | 12:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5, 9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 | 22:1 | 22:2 |
|-----------------|------|------|------|------|-------|---------------|-----------|------------|------------------|-------------------|------|------|------|------|------|
| 5547-LP30108-1 | 0.0 | 5.38 | 0.3 | 2.23 | 64.12 | 0.01 | 0 | 22.67 | 0.44 | 2.17 | 0.07 | 0.82 | 1.11 | 0.03 | 0 |
| 5547-LP30108-2 | 0.1 | 4.45 | 0.13 | 2.29 | 51.57 | 0.16 | 0 | 33.85 | 3.18 | 1.74 | 0.03 | 0.78 | 1.02 | 0.03 | 0.02 |
| 5547-LP30108-3 | 0.0 | 4.18 | 0.12 | 2.03 | 59.61 | 0.03 | 0 | 29.44 | 0.44 | 1.64 | 0 | 0.75 | 1.15 | 0.03 | 0.01 |
| 5547-LP30108-4 | 0.0 | 4.35 | 0.15 | 2.29 | 50.59 | 0.12 | 0.01 | 37.31 | 0.85 | 1.86 | 0.02 | 0.78 | 1.02 | 0.02 | 0.01 |
| 5547-LP30108-5 | 0.0 | 4.59 | 0.14 | 1.83 | 49 | 0.25 | 0.01 | 31.65 | 8.16 | 1.86 | 0.13 | 0.68 | 1.04 | 0.02 | 0 |
| 5547-LP30108-6 | 0.0 | 4.11 | 0.15 | 2.53 | 44.3 | 0.13 | 0 | 28.12 | 15.89 | 1.94 | 0.28 | 0.82 | 1.13 | 0 | 0 |
| 5547-LP30108-7 | 0.0 | 4.27 | 0.15 | 2.55 | 39.18 | 0.12 | 0.02 | 27 | 21.72 | 1.87 | 0.45 | 0.89 | 1.08 | 0 | 0 |
| 5547-LP30108-8 | 0.0 | 4.3 | 0.14 | 2.92 | 42.83 | 0.26 | 0 | 30.81 | 14.51 | 1.49 | 0.22 | 0.89 | 1.06 | 0 | 0 |
| 5547-LP30108-9 | 0.0 | 4.46 | 0.17 | 3.13 | 44.51 | 0 | 0 | 30.12 | 12.87 | 1.76 | 0.22 | 0.98 | 1.12 | 0.01 | 0 |
| 5547-LP30108-10 | 0.0 | 4.28 | 0.11 | 2.62 | 41.44 | 0.28 | 0 | 30.89 | 16.28 | 1.45 | 0.21 | 0.82 | 1.06 | 0 | 0 |
| 5547-LP30108-11 | 0.0 | 4.47 | 0.17 | 2.43 | 26.96 | 0.48 | 0 | 34.44 | 25.01 | 2.14 | 0.63 | 0.84 | 0.99 | 0 | 0 |
| 5547-LP30108-12 | 0.0 | 4.36 | 0.16 | 2.68 | 42.2 | 0.17 | 0 | 29.78 | 15.93 | 1.83 | 0.27 | 0.88 | 1.06 | 0 | 0 |
| 5547-LP30108-13 | 0.0 | 4.87 | 0.19 | 2.81 | 21.7 | 0.53 | 0 | 32.83 | 30.54 | 2.04 | 0.8 | 1 | 0.89 | 0.02 | 0.01 |
| 5547-LP30108-14 | 0.0 | 4.61 | 0.25 | 2.6 | 54 | 0 | 0 | 32.98 | 0.5 | 2.46 | 0.03 | 0.86 | 1.14 | 0 | 0 |
| 5547-LP30108-15 | 0.0 | 4.07 | 0.14 | 2.98 | 37.09 | 0.14 | 0.01 | 29.01 | 21.55 | 1.66 | 0.38 | 1.06 | 1.11 | 0 | 0 |

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

| STRAIN ID | 12:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ5, 9 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 15 | 18:4 | 20:0 | 20:1 | 22:1 | 22:2 |
|-----------------|------|------|------|------|-------|---------------|-----------|------------|------------------|-------------------|------|------|------|------|------|
| 5547-LP30108-16 | 0.0 | 3.63 | 0.13 | 2.12 | 64.69 | 0 | 0 | 24.21 | 0.15 | 2.04 | 0 | 0.82 | 1.56 | 0.02 | 0 |
| 5547-LP30108-17 | 0.0 | 3.85 | 0.18 | 2.22 | 67.22 | 0.01 | 0 | 21.25 | 0 | 2.27 | 0 | 0.83 | 1.53 | 0 | 0 |
| 5547-LP30108-18 | 0.0 | 5.46 | 0.19 | 2.87 | 41.83 | 0.1 | 0.04 | 22.76 | 21.45 | 1.72 | 0.48 | 1.06 | 1.23 | 0 | 0 |
| 5547-LP30108-19 | 0.0 | 4.33 | 0.12 | 2.73 | 50.31 | 0.07 | 0 | 24.77 | 12.72 | 1.62 | 0.21 | 1.04 | 1.29 | 0 | 0.01 |
| 5547-LP30108-20 | 0.0 | 4.22 | 0.12 | 2.91 | 46.33 | 0.25 | 0 | 26.87 | 14.65 | 1.61 | 0.22 | 0.98 | 1.18 | 0 | 0 |
| 5547-LP30108-21 | 0.0 | 4.38 | 0.17 | 2.37 | 55.37 | 0 | 0 | 32.59 | 0.53 | 1.85 | 0.03 | 0.83 | 1.23 | 0 | 0 |
| 5547-LP30108-22 | 0.0 | 5.5 | 0.18 | 2.71 | 41.93 | 0.1 | 0.19 | 24.19 | 20.14 | 1.76 | 0.45 | 0.94 | 1.21 | 0 | 0 |
| 5547-LP30108-23 | 0.0 | 4.03 | 0.16 | 2.17 | 68.44 | 0 | 0 | 20.09 | 0 | 2.19 | 0.02 | 0.83 | 1.46 | 0 | 0 |
| 5547-LP30108-24 | 0.0 | 4.19 | 0.17 | 2.72 | 49.31 | 0 | 0 | 30.38 | 8.64 | 1.85 | 0.13 | 0.86 | 1.16 | 0 | 0 |
| 5547-LP30108-25 | 0.0 | 4.04 | 0.17 | 2.1 | 70.48 | 0 | 0 | 18.04 | 0.05 | 2.09 | 0 | 0.86 | 1.54 | 0 | 0 |
| 5547-LP30108-26 | 0.0 | 4.74 | 0.22 | 3.2 | 26.74 | 0.33 | 0 | 30.05 | 28.95 | 2.02 | 0.78 | 1.08 | 0.99 | 0 | 0 |
| 5547-LP30108-27 | 0.0 | 4.29 | 0.18 | 2.23 | 52.49 | 0 | 0 | 28.48 | 7.36 | 1.91 | 0.13 | 0.87 | 1.37 | 0 | 0 |
| 5547-LP30108-28 | 0.0 | 4.36 | 0.17 | 3 | 44.35 | 0.2 | 0 | 29.59 | 13.39 | 1.91 | 0.23 | 0.96 | 1.17 | 0 | 0 |
| 5547-LP30108-29 | 0.0 | 4.32 | 0.17 | 2.94 | 52.53 | 0.05 | 0 | 33.88 | 0.91 | 2.34 | 0.01 | 0.97 | 1.23 | 0 | 0 |
| 5547-LP30108-30 | 0.0 | 4.07 | 0.14 | 2.89 | 45.13 | 0.01 | 0 | 29.06 | 13.96 | 1.71 | 0.2 | 0.94 | 1.2 | 0.01 | 0 |

Example 13

Stereospecific Distribution of $\Delta 6$ -Desaturated Oils

This experiment was designed to investigate the stereospecific distribution of the $\Delta 6$ -desaturated oils in seeds expressing pCGN5538 (Ma 524 cDNA). Three seed samples were used:

- 1) Non-transformed *B. napus* cv. LP004 seeds (control)
- 2) Segregating T2 seeds of pCGN5538-LP004-19
- 3) Segregating T2 seeds of pCGN5538-LP004-29

The following protocol was used for the analysis:

1. Seed Oil Extraction

Fifty seeds were placed in a 12 x 32 mm vial and crushed with a glass rod. 1.25 mL hexane was added and the mixture was vortexed. The seeds were extracted overnight on a shaker. The extract was then filtered through a 0.2 micron filter attached to a 1cc syringe. The extract was then dried down under nitrogen. The resulting oil was used for digestion and derivatization of the whole oil sample.

2. Digestion

A. Liquid Oil Digestion

The stock lipase (from *Rhizopus arrhizus*, Sigma, L4384) was diluted to approximately 600,000 units/mL with a goal of obtaining 50% digestion of the TAG. The stock lipase is maintained at 4 degrees C and placed on ice. The amount of reagents may be adjusted according to the amount of oil to be digested.

The following amounts are based on a 2.0 mg extracted oil sample. In a 12 x 32 mm screw cap vial the following were added: 2.0 mg oil, 200 μ L 0.1 M tris HCl pH 7, 40 μ L 2.2 w/v% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 100 μ L 0.05 w/v % bile salts. The material was vortexed and sonicated to disperse the oil. Twenty μ L of diluted lipase was added and the mixture was vortexed continuously for 1.0

minute at room temperature. A white precipitate formed. The reaction was stopped with 100 μ L 6M HCl and vortexing. Five hundred μ L $\text{CHCl}_3\text{:CH}_3\text{OH}$ (2:1) was added and the mixture was vortexed and held on ice while reaining digestions were carried out. Samples were vortexed again and centrifuged briefly to sharpen layers. The lower layer containing digest products was removed with a pasteur pipette and placed in a 12 x 32 mm crimp cap vial. The material was then re-extracted with 300 μ L CHCl_3 , vortexed, centrifuged, and combined with the lower layers. The digest products were kept on ice as much as possible. HPLC separation is performed as soon as possible after digestion to minimize acyl migration.

B. Solid Fat Digestion

The procedure for liquid oil digestion described above was followed except that 20 μ l 11:0 methyl ester is added to 2.0 mg solid fat.

3. HPLC Separation

The digestion products were dried down in chloroform to approximately 200 μ L. Each sample was then transferred into an insert in an 8 x 40 mm shell vial and 30 μ L was injected for HPLC analysis.

The high performance liquid chromatographic system was equipped with a Varex ELSD IIA evaporative light scattering detector with tube temperature at 105°C and nitrogen gas flow at 40 mL/min; a Waters 712 Wisp autosampler, three Beckman 114M Solvent Delivery Modules; a Beckman 421A controller, a Rheodyne pneumatically actuated stream splitter; and a Gilson micro fractionator. The chromatography column is a 220 x 4.6 mm, 5 micron normal phase silica cartridge by Brownlee.

The three solvents used were:

A= hexane:toluene 1:1

B= toluene: ethyl acetate 3:1

C= 5% formic acid in ethyl acetate

The gradient profile was as follows:

| Time (min) | Function | Value | Duration |
|------------|-------------|-------|----------|
| 0 flow | 2.0 mL/min | | |
| 0 % B | 10 | | |
| 0 % C | 2 | | |
| 2 % C | 25 | | 6 min |
| 14.0 % C | 2 | | 1 min |
| 15.0 | End program | | |

A chromatographic standard mixture is prepared in hexane:toluene 1:1 containing the following:

- 0.2 mg/mL triglyceride 16:0
- 5 2.0 mg/mL 16:0 Free Fatty Acid
- 0.2 mg/mL di16:0 mixed isomers (1,2-diacylglycerol and 1,3-diacylglycerol)
- 0.2 mg/mL 3-mono acylglycerol 16:0
- 0.2 mg/mL 2-mono acylglycerol 16:0

10 For each sample, the fraction containing the 2-mag peak is collected automatically by method controlled timed events relays. A time delay is used to synchronize the detector with the collector's emitter. The 2-mag peaks are collected and the fractions are evaporated at room temperature overnight.

The *sn*-2 composition results rely on minimization of acyl migration. Appearance of 1-monoacylglycerol and/or 3-monoacylglycerol peaks in the chromatograph means that acyl migration has occurred.

4. Derivatization

20 To derivatize the whole oil, 1.0 mg of the extracted whole oil was weighed into a 12 x 32 mm crimp cap vial. One mL toluene was then added. The sample is then vortexed and a 50 μ L aliquot was removed for derivatization. To the dried down 2-mag samples, 50 μ L toluene was added. To both the whole oil and 2-mag fractions 105 μ L H₂SO₄/CH₃OH @ 8.76 wt% is added. The cap was tightly capped and the sample is refluxed for 1 hour at 95 degrees C. The sample was allowed to cool and 500 μ L 10 w/v % NaCl in

water and 60 uL heptane was added. The organic layer was removed and inserted in a 12 x 32 mm crimp cap vial.

5. GLC Analysis

5 A Hewlett Packard model 6890 GC equipped with a split/splitless capillary inlet, FID detector, 6890 series autosampler and 3392A Alpha Omega integrator is set up for the capillary column as follows:

A. Supelco Omegawax 250, 30 m length, 0.25 mm id, 0.25 um film thickness

| | | |
|----|-----------------|----------------|
| 10 | injection port: | 260 C |
| | detector: | 270 C |
| | initial temp: | 170 C |
| | initial time: | 1.5 min |
| | rate: | 30 deg/min |
| 15 | final temp: | 245 C |
| | final time: | 6.5 min |
| | injection vol: | 1.5 uL |
| | head pressure: | 25 psi |
| | split ratio: | 30 |
| 20 | carrier gas: | He |
| | make-up gas: | N ₂ |
| | FID gas: | H + air |

Percent compositions of fatty acid methyl esters are calculated as mole percents. For carbon chain lengths less than 12, the use of theoretical or
25 empirical response factors in the area percent calculation is desirable.

6. Calculations

The mean distribution of each acyl group at each *sn*-1 and *sn*-3 position was calculated.

mean *sn*-1 and *sn*-3 composition = (3 WO comp - MAG comp) / 2

5 WO = whole oil

MAG= monoacylglycerol

The results of this analysis are presented in Table 14. The GLA and $\Delta^{6,9}$ 18:2 are evenly distributed between the *sn*-2 and *sn*-1, 3 positions. This analysis can not discriminate between fatty acids in the *sn*-1 vs. *sn*-3 positions.

Table 14

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2 | 18:3_Δ6,9,12 | 8:3 | 18:4 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|-------|--------------|------|------|------|------|
| Control | | | | | | | | | | | |
| sn2 composition | 1.23 | 0.15 | 0.37 | 64.77 | 0.00 | 29.45 | 0.06 | 2.01 | 0.00 | 0.21 | 0.57 |
| whole oil composition | 4.33 | 0.20 | 3.32 | 69.29 | 0.18 | 18.51 | 0.00 | 1.35 | 0.06 | 0.91 | 1.17 |
| mean sn1, sn3 composition* | 5.88 | 0.23 | 4.80 | 71.55 | 0.27 | 13.04 | -0.03 | 1.02 | 0.09 | 1.26 | 1.47 |
| | | | | | | | | | | | |
| 5538-19 | | | | | | | | | | | |
| sn2 composition | 1.65 | 0.27 | 4.12 | 57.21 | 5.61 | 14.55 | 12.45 | 1.38 | 0.32 | 0.43 | 1.00 |
| whole oil composition | 5.44 | 0.33 | 4.09 | 57.51 | 4.53 | 10.57 | 13.16 | 1.03 | 0.50 | 1.07 | 1.07 |
| mean sn1, sn3 composition* | 7.34 | 0.36 | 4.08 | 57.66 | 3.99 | 8.58 | 13.52 | 0.86 | 0.59 | 1.39 | 1.11 |
| | | | | | | | | | | | |
| 5538-29 | | | | | | | | | | | |
| sn2 composition | 1.24 | 0.27 | 1.56 | 56.35 | 6.35 | 17.85 | 12.99 | 1.60 | 0.38 | 0.14 | 0.40 |
| whole oil composition | 4.96 | 0.32 | 3.73 | 54.92 | 4.99 | 12.11 | 13.66 | 1.10 | 0.50 | 0.99 | 1.11 |
| mean sn1, sn3 composition* | 6.82 | 0.35 | 4.82 | 54.21 | 4.31 | 9.24 | 14.00 | 0.85 | 0.56 | 1.42 | 1.47 |
| | | | | | | | | | | | |
| *calculated from the mag and whole oil composition for each analyte | | | | | | | | | | | |

Example 14

Fatty Acid Compositions of Transgenic Plants

$\Delta 5$ and $\Delta 6$ transgenic plants were analyzed for their fatty acid content.

The following protocol was used for oil extraction:

- 5 1. About 400 mg of seed were weighed out in duplicate for each sample.
2. The seeds were crushed in a mortar and pestle. The mortar and pestle was rinsed twice with 3ml (2:1) (v:v) CHCl_3 : CH_3OH /MeOH. An additional 6 ml (2:1) was added to
10 the 20ml glass vial (oil extracted in 12ml total 2:1).
3. Samples were vortexed and placed on an orbital shaker for 2 hours with occasional vortexing.
4. 5ml of 1M NaCl was added to each sample. Sample was vortexed then spun in centrifuge at 2000rpm for 5 minutes.
15 Lower phase was drawn off using a pasteur pipette.
5. Upper phase was re-extracted with an additional 5ml. Sample was vortexed then spun in centrifuge at 2000 rpm for 5 minutes. The lower phase was drawn off using a pasteur pipette and added to previous lower phase.
- 20 6. CHCl_3 : CH_3OH /MeOH was evaporated under nitrogen using evaporative cooling. Vial containing extracted oil was sealed under nitrogen. Between 120mg- 160mg oil was extracted for each sample.

25 For GC-MS analysis, fatty acid methyl esters were dissolved in an appropriate volume of hexane and analyzed using a Hewlett-Packard 5890 Series II Plus gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a 30 m x 0.32 mm i.d. Omegawax 320 fused silica capillary column (Supelco, Bellefonte, PA) and a Hewlett-Packard 5972 Series mass selective detector. Mass spectra were interpreted by comparison to the mass spectra in

NIST/EPA/NIH Chemical Structure Database using a MS Chem Station (#G1036A) (Hewlett Packard).

Transgenic line 5531-6 was analyzed in duplicate (A, B) and compared to control line LP004-6. The fatty acid profile results are shown in Table 15.

- 5 Transgenic line 5538-19 was analyzed in duplicate (A, B) and compared to control line LP004-6. The fatty acid profile results are shown in Table 16.

Table 15
Fatty Acid Profile

| | CONTROL | CONTROL | TRANSGENIC | TRANSGENIC |
|--------------|------------|------------|------------|------------|
| | LP004-6A | LP004-6B | 5531-6A | 5531-6B |
| | LRL-2043 | LRL-2044 | LRL-2042 | LRL-2045 |
| | 001f0102.d | 001f0103.d | 001f0101.d | 001f0104.d |
| C12:0 | | | | |
| C13:0 | | | | |
| C14:0 | | 0.053 | | 0.061 |
| C14:1 | | | | |
| C15:0 isomer | | | | |
| C15:0 | | | | |
| C16:0 | 4.107 | 4.034 | 4.257 | 4.224 |
| C16:1 | 0.181 | 0.173 | 0.200 | 0.199 |
| C16:2 | 0.061 | 0.065 | 0.081 | 0.060 |
| C17:0 | | | | |
| C16:3 | 0.244 | 0.246 | 0.155 | 0.151 |
| C16:4 | | | | |
| C18:0 | 2.608 | 2.714 | 3.368 | 3.417 |
| C18:1w9 | 65.489 | 66.454 | 59.529 | 59.073 |
| C18:1w7 | 2.297 | 2.185 | 2.388 | 2.393 |
| C18:2 5,9 | | | 6.144 | 6.269 |
| C18:2w6 | 19.828 | 18.667 | 18.872 | 19.059 |
| C18:3 5,9,12 | | | 0.469 | 0.496 |
| C18:3w6 | | 0.060 | | |
| C18:3w3 | 1.587 | 1.578 | 1.428 | 1.418 |
| C18:4w6 | | | | |
| C18:4w3 | | | | |
| C20:0 | 0.962 | 0.998 | 1.009 | 1.022 |
| C20:1w11 | 1.336 | 1.335 | 1.058 | 1.065 |
| C20:1w9 | | | | |
| C20:1w7 | | | 0.076 | 0.080 |
| C20:2w6 | 0.073 | 0.073 | | 0.052 |
| C20:3w6 | | | | |

Table 15
Fatty Acid Profile

| | CONTROL | CONTROL | TRANSGENIC | TRANSGENIC |
|---------------------|-------------------|-------------------|-------------------|-------------------|
| | | | | |
| | LP004-6A | LP004-6B | 5531-6A | 5531-6B |
| | | | | |
| | LRL-2043 | LRL-2044 | LRL-2042 | LRL-2045 |
| | 001f0102.d | 001f0103.d | 001f0101.d | 001f0104.d |
| C20:4w6 | | | | |
| C20:3w3 | | | | |
| C20:4w3 | | | | |
| C20:5w3 | | | | |
| C22:0(1.000) | 0.542 | 0.558 | 0.463 | 0.467 |
| C22:1w11 | | 0.038 | | |
| C22:1w9 | | | | |
| C22:1w7 | | 0.034 | | |
| C21:5 | | | | |
| C23:0 | | 0.029 | | |
| C22:4w6 | | | | |
| C22:5w6 | | | | |
| C22:5w3 | | | | |
| C24:0 | 0.373 | 0.391 | 0.280 | 0.283 |
| C22:6w3 | 0.314 | 0.317 | 0.223 | 0.212 |
| C24:1w9 | | | | |
| | | | | |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 |

Table 16
Fatty Acid Profile

| | 5538-19A | 5538-19B | LP004-6A | LP004-6B |
|-------------|------------|------------|----------|----------|
| | TRANSGENIC | TRANSGENIC | CONTROL | CONTROL |
| | LRL-2166 | LRL-2167 | LRL-2168 | LRL-2169 |
| C6:0 | 0.004 | 0.005 | | |
| C8:0 | 0.007 | 0.007 | 0.004 | 0.005 |
| C10:0 | 0.012 | 0.012 | 0.008 | 0.008 |
| C12:0 | 0.020 | 0.020 | 0.011 | 0.012 |
| C13:0 | | | | |
| C14:0 | 0.099 | 0.108 | 0.050 | 0.050 |
| C14:1w5 | | | | |
| C15:0 | 0.059 | 0.068 | 0.017 | 0.019 |
| C16:0 | 5.272 | 5.294 | 4.049 | 4.057 |
| C16:1 | 0.350 | 0.417 | 0.197 | 0.208 |
| C16:2 | 0.199 | 0.187 | 0.076 | 0.077 |
| C17:0 | 0.092 | 0.089 | 0.078 | 0.077 |
| C16:3 | 0.149 | 0.149 | 0.192 | 0.198 |
| C16:4 | | 0.010 | | |
| C18:0 | 3.815 | 3.771 | 2.585 | 2.638 |
| C18:1 | 57.562 | 57.051 | 68.506 | 68.352 |
| C18:2 (6,9) | 4.246 | 4.022 | | |
| C18:2w6 | 10.900 | 11.589 | 19.098 | 19.122 |
| C18:2w3 | 0.020 | 0.008 | 0.008 | 0.009 |
| C18:3w6 | 12.565 | 12.595 | 0.013 | 0.015 |
| C18:3w3 | 1.084 | 1.137 | 1.501 | 1.542 |
| C18:4 | 0.017 | 0.013 | 0.011 | 0.008 |
| C18:4 | 0.028 | 0.024 | | |
| C20:0 | 1.138 | 1.104 | 0.937 | 0.943 |
| C20:1 | 1.115 | 1.085 | 1.330 | 1.327 |
| C20:2w6 | 0.150 | 0.143 | 0.068 | 0.071 |
| C20:3w6 | 0.026 | 0.025 | 0.014 | 0.012 |
| C20:4w6 | | | | |
| C20:3w3 | | | | |

Table 16
Fatty Acid Profile

| | 5538-19A | 5538-19B | LP004-6A | LP004-6B |
|----------------|-------------------|-------------------|-----------------|-----------------|
| | TRANSGENIC | TRANSGENIC | CONTROL | CONTROL |
| | | | | |
| | LRL-2166 | LRL-2167 | LRL-2168 | LRL-2169 |
| | | | | |
| C20:4w3 | | | | |
| C20:5w3 | | | | |
| C22:0 | 0.506 | 0.484 | 0.535 | 0.539 |
| C22:1 | 0.017 | 0.020 | 0.032 | 0.032 |
| C21:5 | | 0.040 | 0.030 | 0.031 |
| C22:4w6 | 0.038 | 0.064 | 0.015 | 0.014 |
| C22:5w6 | | | | |
| C22:5w3 | 0.023 | 0.018 | 0.021 | 0.017 |
| C24:0 | 0.352 | 0.321 | 0.353 | 0.362 |
| C22:6w3 | 0.009 | | | |
| C24:1w9 | 0.129 | 0.121 | 0.260 | 0.255 |
| | | | | |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 |

Example 15

Combined Expression of $\Delta 6$ and $\Delta 12$ Desaturases in *B. napus* Achieved by Crossing

Plants containing either the $\Delta 6$ or the $\Delta 12$ desaturase were crossed and individual F1 half-seeds were analyzed for fatty acid composition by GC. Data from one such cross are given in Table 17. The parents for the cross were 5538-LP004-25-2-25 ($\Delta 6$ expressor) and 5542-SP30021-10-16 ($\Delta 12$ expressor). Reciprocal crosses were made and the results of 25 individual F1 seeds of each are shown in the table. Crosses are described such that the first parent indicated is the female. Both sets of crosses gave approximately the same results. Compared to the parents, the $\Delta^{6,9}$ 18:2 decreased, and the GLA increased. $\Delta^{9,12}$ 18:2 levels are increased in most of the F1's as well. Note that these are F1 seeds and only contain one set of each desaturase. In future generations and selection of events homozygous for each desaturase, the F2 GLA levels obtained may be even higher.

Combining traits by crossing may be preferable to combining traits on one T-DNA in some situations. Particularly if both genes are driven off of the same promoter (in this case napin), issues of promoter silencing may favor this approach over putting multiple cDNAs on one construct.

Alternatively, in some cases, combining multiple cDNAs on one T-DNA may be the method of choice. The results are shown in Table 17.

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|
| 5538-LP004-25-2-25 | 4.23 | 0.13 | 2.4 | 61.78 | 8.77 | 6.34 | 11.58 | 0.92 | 0 | 0 |
| 5542-SP30021-10-16 | 4.09 | 0.1 | 2.03 | 38.4 | 0 | 41.88 | 0 | 11.06 | 0.02 | 0.75 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.9 | 0.04 | 2.31 | 38.58 | 0 | 27.91 | 20.94 | 2.67 | 0.65 | 0.92 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.5 | 0.04 | 1.88 | 36.24 | 0 | 28.68 | 22.54 | 3.36 | 0.85 | 0.78 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.51 | 0.03 | 1.98 | 38.36 | 0 | 29.48 | 19.95 | 3.06 | 0.68 | 0.79 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.95 | 0.04 | 1.86 | 38.65 | 0 | 28.08 | 20.81 | 2.92 | 0.75 | 0.76 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.26 | 0.05 | 2.44 | 40.25 | 0.01 | 28.81 | 18.08 | 2.74 | 0.53 | 0.88 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.13 | 0.04 | 2.33 | 34.48 | 0 | 26.73 | 26.2 | 2.32 | 0.75 | 0.9 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.8 | 0.04 | 2.15 | 38.34 | 0 | 28.95 | 20.64 | 2.63 | 0.65 | 0.81 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.96 | 0.05 | 1.59 | 36.43 | 0 | 29.05 | 21.85 | 3.47 | 0.86 | 0.68 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.04 | 0.04 | 2.5 | 37.75 | 0 | 27.23 | 22.89 | 1.95 | 0.55 | 0.99 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.53 | 0.04 | 1.8 | 34.88 | 0 | 29.17 | 23.42 | 3.42 | 0.9 | 0.74 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.43 | 0.04 | 1.89 | 37.12 | 0 | 29.52 | 20.91 | 3.35 | 0.8 | 0.79 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.58 | 0.03 | 2.55 | 39.54 | 0 | 28.81 | 19.34 | 2.44 | 0.54 | 0.98 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.53 | 0.03 | 2.33 | 39.26 | 0 | 29.07 | 19.5 | 2.61 | 0.59 | 0.91 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.4 | 0.02 | 2.41 | 45.53 | 0 | 28.94 | 13.71 | 2.51 | 0.37 | 0.91 |

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 18:4 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.49 | 0.03 | 2.57 | 40.95 | 0 | 28.52 | 17.97 | 2.63 | 0.58 | 0.99 | 1.43 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.65 | 0.04 | 2.11 | 38.02 | 0 | 29.13 | 20.53 | 2.85 | 0.66 | 0.86 | 1.33 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.97 | 0.03 | 1.99 | 34.95 | 0.01 | 27.15 | 25.71 | 2.38 | 0.79 | 0.81 | 1.38 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.81 | 0.05 | 1.46 | 38.3 | 0 | 31.51 | 17.67 | 3.83 | 0.75 | 0.61 | 1.33 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.98 | 0.05 | 2.03 | 37.14 | 0 | 30.09 | 20.28 | 2.79 | 0.72 | 0.8 | 1.36 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.03 | 0.04 | 2.52 | 42.9 | 0 | 27.79 | 16.66 | 2.64 | 0.54 | 0.9 | 1.29 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.03 | 0.04 | 2.27 | 40.72 | 0 | 29.37 | 17.56 | 2.53 | 0.53 | 0.86 | 1.35 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.98 | 0.04 | 2.61 | 39.91 | 0 | 28.06 | 19.15 | 2.69 | 0.6 | 0.96 | 1.26 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 3.73 | 0.03 | 1.89 | 40.22 | 0 | 29.44 | 18.21 | 3 | 0.67 | 0.73 | 1.39 |
| (5538-LP004-25-2-25 X 5542-SP30021-10-16) | 4.02 | 0.04 | 2.14 | 42.58 | 0 | 30.36 | 15.18 | 2.43 | 0.42 | 0.82 | 1.3 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.14 | 0.06 | 2.23 | 30.67 | 0 | 30.38 | 25.47 | 3.12 | 0.91 | 0.9 | 1.29 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.05 | 0.07 | 1.7 | 37.03 | 0.04 | 32.1 | 15.97 | 5.38 | 0.96 | 0.69 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.01 | 0.07 | 1.58 | 38.02 | 0.05 | 33.65 | 13.92 | 5.15 | 0.89 | 0.66 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.07 | 0.06 | 2.01 | 31.63 | 0.05 | 31.13 | 23.09 | 3.94 | 1.1 | 0.83 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.03 | 0.05 | 1.94 | 31.88 | 0 | 30.98 | 23.71 | 3.45 | 0.99 | 0.82 | 1.3 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.92 | 0.06 | 1.71 | 35.77 | 0.03 | 33.15 | 16.39 | 5.28 | 0.98 | 0.68 | 1.32 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.09 | 0.08 | 1.57 | 34.6 | 0.03 | 33.73 | 16.73 | 5.48 | 0.99 | 0.66 | 1.28 |

Table 17

| STRAIN ID | 16:0 | 16:1 | 18:0 | 18:1 | 18:2_Δ6,9 | 18:2_Δ9,12 | 18:3_Δ6,9, 12 | 18:3_Δ9,12, 11 | 18:4 | 20:0 | 20:1 |
|---|------|------|------|-------|-----------|------------|------------------|-------------------|------|------|------|
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.94 | 0.07 | 1.59 | 34.03 | 0.04 | 31.35 | 19.76 | 5.29 | 1.22 | 0.67 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.13 | 0.06 | 1.85 | 31.44 | 0.06 | 31.28 | 23.77 | 3.52 | 1.04 | 0.79 | 1.22 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.14 | 0.06 | 1.96 | 31.11 | 0.04 | 31.88 | 23.3 | 3.6 | 1.01 | 0.82 | 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.98 | 0.07 | 1.58 | 35.06 | 0 | 32.06 | 18.1 | 5.33 | 1.12 | 0.67 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.89 | 0.06 | 1.59 | 32.51 | 0.05 | 29.44 | 22.91 | 5.33 | 1.54 | 0.67 | 1.25 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4 | 0.07 | 1.69 | 32.1 | 0.05 | 30.49 | 22.77 | 4.66 | 1.32 | 0.75 | 1.26 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.06 | 0.05 | 1.93 | 30.77 | 0.07 | 28.37 | 27.21 | 3.37 | 1.19 | 0.84 | 1.25 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.1 | 0.06 | 1.9 | 31.77 | 0.05 | 32.33 | 22.03 | 3.92 | 0.98 | 0.78 | 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.94 | 0.07 | 1.67 | 34.74 | 0.03 | 33.63 | 17.1 | 5.16 | 0.99 | 0.68 | 1.26 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.71 | 0.06 | 1.65 | 33.05 | 0 | 33.22 | 19.73 | 4.7 | 1.07 | 0.68 | 1.39 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 3.84 | 0.06 | 1.71 | 34.16 | 0.04 | 34.52 | 16.74 | 5.18 | 0.97 | 0.68 | 1.34 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4 | 0.07 | 1.66 | 34.97 | 0.07 | 33.08 | 17.07 | 5.27 | 1.1 | 0.67 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.16 | 0.06 | 1.99 | 35.44 | 0.05 | 31.89 | 18.95 | 3.68 | 0.89 | 0.81 | 1.29 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.05 | 0.08 | 1.46 | 33.49 | 0 | 31.96 | 18.81 | 6.2 | 1.32 | 0.61 | 1.28 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.2 | 0.06 | 1.93 | 35.06 | 0.06 | 33.69 | 17.38 | 4 | 0.86 | 0.78 | 1.21 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.07 | 0.06 | 1.74 | 36 | 0.06 | 32.18 | 17.86 | 4.32 | 0.96 | 0.73 | 1.27 |
| (5542-SP30021-10-16 X 5538-LP004-25-2-25) | 4.11 | 0.05 | 2.24 | 29.64 | 0.04 | 28.64 | 27.94 | 3.06 | 1.12 | 0.97 | 1.26 |

Example 16

Expression of *M. alpina* desaturases in soybean

The *M. alpina* desaturases can be used to drive production of GLA and other PUFAs in soybean by use of the following expression constructs. Two
5 means by which exogenous DNA can be inserted into the soybean genome are *Agrobacterium* infection or particle gun. Particle gun transformation is disclosed in U.S. patent 5,503,998. Plants can be selected using a glyphosate resistance marker (4, 971, 908). *Agrobacterium* transformation of soybean is well established to one of ordinary skill in the art.

10 For seed specific expression, the coding regions of the desaturase cDNAs are placed under control of the 5' regulatory region of *Glycine max* alpha-type beta conglycinin storage protein gene. The specific region that can be used is nucleotides 78-921 of gi 169928 (Doyle, J.J., Schuler, M.A., Godette, W.D., Zenger, V., Beachy, R.N., and Slightom, J.L., 1986 J. Biol.
15 Chem. 261 (20), 9228-9238). The 3' regulatory region that can be used is from the pea ribulose 1,5 biphosphate carboxylase/oxygenase small subunit (rbcS) gene. The specific sequences to be used are nucleotides 1-645 of gi 169145 (Hunt, A.G. 1988 DNA 7: 329-336).

20 Since soybean seeds contain more 18:2, and perhaps more endogenous $\Delta 12$ desaturase activity than *Brassica*, the effect of the *Mortierella* $\Delta 12$ desaturase on achieving optimal GLA levels can be tested as follows. A construct containing the $\Delta 6$ cDNA can be used to see if $\Delta^{6,9}$ 18:2 is produced along with GLA. A construct containing the $\Delta 12$ desaturase can be used to see if the amount of 18:2 can be increased in soybean. A construct containing both
25 the $\Delta 6$ and $\Delta 12$ desaturases can be used to produce optimal levels of GLA. Alternatively, plants containing each of the single desaturases may be crossed if necessary to combine the genes.

Similar constructs may be made to express the $\Delta 5$ desaturase alone, or in combination with $\Delta 12$ and/or $\Delta 6$ desaturases.

Example 17

Human Desaturase Gene Sequences

Human desaturase gene sequences potentially involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology
5 between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases were found. As with some other membrane-bound desaturases the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases
10 exhibited homology to *M. alpina* $\Delta 5$, $\Delta 6$, $\Delta 9$, and $\Delta 12$ desaturases.

The *M. alpina* $\Delta 5$ desaturase and $\Delta 6$ desaturase cDNA sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, California 94304. The $\Delta 5$ desaturase sequence was divided into fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-
15 446. The $\Delta 6$ desaturase sequence was divided into three fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames
20 (both strands).

The polypeptide fragments 2 and 3 of *M. alpina* $\Delta 5$ and $\Delta 6$ have homologies with the CloneID sequences as outlined in Table 18. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results have been reviewed, Clone Information was searched with the
25 default settings of Stringency of ≥ 50 , and Productscore ≤ 100 for different CloneID numbers. The Clone Information Results displayed the information including the ClusterID, CloneID, Library, HitID, Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command assembles all of the
30 CloneID which comprise the ClusterID. The following default settings were

used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, Wisconsin 53705) Assembly:

| | | |
|----|-------------------|-----|
| | Word Size: | 7 |
| 5 | Minimum Overlap: | 14 |
| | Stringency: | 0.8 |
| | Minimum Identity: | 14 |
| | Maximum Gap: | 10 |
| | Gap Weight: | 8 |
| 10 | Length Weight: | 2 |

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequences within a contig. The contig containing the CloneID was identified, and the ambiguous sites of the consensus sequence was edited based on the alignment of the CloneIDs (see SEQ ID NO:31 - SEQ ID NO:35) to generate the best possible sequence. The procedure was repeated for all six CloneID listed in Table 18. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan 48105). These consensus sequences were assembled. The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (SEQ ID NO:37). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into protein sequences. The *M. alpina* $\Delta 5$ (MA29) and $\Delta 6$ (MA524) sequences were compared with each of the translated contigs using the FastA search (a Pearson

and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig. The homology among the *M. alpina* $\Delta 5$ and $\Delta 6$ to contigs 2535 and 3854933 were utilized to create the final contig called 253538a. Figure 9 is the FastA match of the final contig 253538a and MA29, and Figure 10 is the FastA match of the final contig 253538a and MA524. The DNA sequences for the various contigs are presented in SEQ ID NO:31 -SEQ ID NO:37 The various peptide sequences are shown in SEQ ID NO:38 - SEQ ID NO: 44.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acids. It starts with Gln (CAG) and ends with the stop codon (TGA). The contig 253538a aligns with both *M. alpina* $\Delta 5$ and $\Delta 6$ sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Table 18, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

Uses of the Human Desaturases

These human sequences can be expressed in yeast and plants utilizing the procedures described in the preceding examples. For expression in mammalian cells and transgenic animals, these genes may provide superior codon bias. In addition, these sequences can be used to isolate related desaturase genes from other organisms.

Table 18

| Sections of the Desaturases | Clone ID from LifeSeq Database | Keyword |
|-----------------------------|--------------------------------|-----------------------|
| 151-300 $\Delta 5$ | 3808675 | fatty acid desaturase |
| 301-446 $\Delta 5$ | 354535 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 3448789 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 1362863 | $\Delta 6$ |
| 151-300 $\Delta 6$ | 2394760 | $\Delta 6$ |
| 301-457 $\Delta 6$ | 3350263 | $\Delta 6$ |

Example 185 **Identification of Homologues to *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases**

A nucleic acid sequence that encodes a putative $\Delta 5$ desaturase was identified through a TBLASTN search of the expressed sequence tag databases through NCBI using amino acids 100-446 of Ma29 as a query. The truncated portion of the Ma29 sequence was used to avoid picking up homologies based on the cytochrome b5 portion at the N-terminus of the desaturase. The deduced amino acid sequence of an est from *Dictyostelium discoideum* (accession # C25549) shows very significant homology to Ma29 and lesser, but still significant homology to Ma524. The DNA sequence is presented as SEQ ID NO:45. The amino acid sequence is presented as SEQ ID NO:46.

15

Example 19**Identification of *M. alpina* $\Delta 5$ and $\Delta 6$ homologues in other PUFA-producing organisms**

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Phaeodactylum tricornutum*. A plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative $\Delta 5$ or $\Delta 6$ desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

One clone was identified from the *Phaeodactylum* library with homology to Ma29 and Ma524; it is called 144-011-B12. The DNA sequence is presented as SEQ ID NO:47. The amino acid sequence is presented as SEQ ID NO:48.

5

Example 20

Identification of *M. alpina* $\Delta 5$ and $\Delta 6$ homologues in other PUFA-producing organisms

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Schizochytrium* species. A plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative $\Delta 5$ or $\Delta 6$ desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

15

One clone was identified from the *Schizochytrium* library with homology to Ma29 and Ma524; it is called 81-23-C7. This clone contains a ~1 kb insert. Partial sequence was obtained from each end of the clone using the universal forward and reverse sequencing primers. The DNA sequence from the forward primer is presented as SEQ ID NO:49. The peptide sequence is presented as SEQ ID NO:50. The DNA sequence from the reverse primer is presented as SEQ ID NO:51. The amino acid sequence from the reverse primer is presented as SEQ ID NO:52.

20

Example 21

Nutritional Compositions

25

The PUFAs of the previous examples can be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutrition solutions.

I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron.

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

5

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity
- Lactose-free formulation to avoid lactose-associated diarrhea
- Low osmolality (240 mOsm/kg water) to reduce risk of osmotic diarrhea.

10

- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.

15

- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

20

Ingredients: (Pareve, ®) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic

25

acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea.

5 Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- 10 • Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- 15 • Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 20 • Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- 25 • Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve, ©) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy

fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono- and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

10 C. Isomil® SF Sucrose-Free Soy Formula With Iron.

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- 15 • Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- 20 • Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- 25 • Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve, ®) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1% soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch,

0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride, carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

**D. Isomil® 20 Soy Formula With Iron Ready To Feed,
20 Cal/fl oz.**

Usage: When a soy feeding is desired.

Ingredients: (Pareve, ®) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

E. Similac® Infant Formula

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

Features:

- Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
- 5 • Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
- Carbohydrate as lactose in proportion similar to that of human milk.
- Low renal solute load to minimize stress on developing organs.
- 10 • Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (©-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamid, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, 15 thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron

Usage: For premature infants' special nutritional needs after hospital 20 discharge. Similac NeoCare is a nutritionally complete formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

- 25 • Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 Calories to extend the nutritional support initiated in-hospital.

- More calcium and phosphorus for improved bone mineralization.

Ingredients: @-D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium-chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D₃ and cyanocobalamin.

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: @-D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soil oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono- and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D₃, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art..

II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

Ingredients:

®-D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-

rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:

- 5
- For patients who need extra calories, protein, vitamins and minerals
 - Especially useful for people who do not take in enough calories and nutrients
 - For people who have the ability to chew and swallow
 - Not to be used by anyone with a peanut allergy or any type of allergy to nuts.
- 10

Ingredients:

- Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein-Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.
- 15

20 **Vitamins and Minerals:**

- Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta-Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.
- 25

Protein:

Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

| | | |
|---|---------------------|-----|
| | Soy protein isolate | 74% |
| 5 | Milk proteins | 26% |

Fat:

Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, and corn oils, and soy lecithin.

| | | |
|----|---|-----|
| 10 | Partially hydrogenated cottonseed and soybean oil | 76% |
| | Canola oil | 8% |
| | High-oleic safflower oil | 8% |
| | Corn oil | 4% |
| | Soy lecithin | 4% |

15 **Carbohydrate:**

Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

| | | |
|----|--------------------------|-----|
| | High-fructose corn syrup | 24% |
| 20 | Brown sugar | 21% |
| | Maltodextrin | 12% |
| | Honey | 11% |
| | Crisp rice | 9% |
| | Glycerine | 9% |
| 25 | Soy polysaccharide | 7% |
| | Oat bran | 7%\ |

C. ENSURE® HIGH PROTEIN

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions

- For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features-

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride,

Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein:

- 5 The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | |
|-------------------------------|-----|
| Sodium and calcium caseinates | 85% |
| Soy protein isolate | 15% |

Fat:

- 10 The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

| | |
|--------------------------|-----|
| High-oleic safflower oil | 40% |
| Canola oil | 30% |
| Soy oil | 30% |

- 15 The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.
- 20

Carbohydrate:

- 25 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORSO® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | |
|---------|-----|
| Sucrose | 60% |
|---------|-----|

| | |
|------------------|-----|
| Maltodextrin | 40% |
| Chocolate | |
| Sucrose | 70% |
| Maltodextrin | 30% |

5

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

10

Patient Conditions:

- For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE
- For healthy adults who don't eat right and need extra nutrition

15

Features:

- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

20

Ingredients:

French Vanilla: ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride),

25

Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein:

The protein source is calcium caseinate.

| | |
|-------------------|------|
| Calcium caseinate | 100% |
|-------------------|------|

10 Fat

The fat source is a blend of two oils: high-oleic safflower and canola.

| | |
|--------------------------|-----|
| High-oleic safflower oil | 70% |
|--------------------------|-----|

| | |
|------------|-----|
| Canola oil | 30% |
|------------|-----|

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.

20 Carbohydrate

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | |
|---------|-----|
| Sucrose | 51% |
|---------|-----|

| | |
|--------------|-----|
| Maltodextrin | 49% |
|--------------|-----|

Chocolate

| | |
|--------------|-------|
| Sucrose | 47.0% |
| Corn Syrup | 26.5% |
| Maltodextrin | 26.5% |

5 **Vitamins and Minerals**

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

10

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

15

Patient Conditions:

- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

20

Features

- Rich, creamy taste
- Good source of essential vitamins and minerals

25

Ingredients

Vanilla: ®-D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride,

Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D₃.

Protein

The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | |
|-------------------------------|-----|
| Sodium and calcium caseinates | 84% |
| Soy protein isolate | 16% |

Fat

The fat source is corn oil.

| | |
|----------|------|
| Corn oil | 100% |
|----------|------|

Carbohydrate

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, butter pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors

| | |
|--------------|-----|
| Corn Syrup | 39% |
| Maltodextrin | 38% |
| Sucrose | 23% |

Chocolate and eggnog flavors

| | |
|------------|-----|
| Corn Syrup | 36% |
|------------|-----|

| | |
|--------------|-----|
| Maltodextrin | 34% |
| Sucrose | 30% |

Vitamins and Minerals

5 An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

10 **F. ENSURE PLUS® HN**

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

15

Patient Conditions:

- For patients with increased calorie and protein needs, such as following surgery or injury
- For patients with limited volume tolerance and early satiety

20 **Features**

- For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaV/mL
- High nitrogen
- 25 • Calorically dense

Ingredients

Vanilla: ©-D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial
5 Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium
10 Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D₃.

G. ENSURE® POWDER

Usage: ENSURE POWDER (reconstituted with water) is a low-residue
15 liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

- For patients on modified diets
- 20 • For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

Features

- Convenient, easy to mix
- 25 • Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets

- Lactose-free, easily digested

Ingredients: ©-D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate

5 Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide,

10 Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein

The protein source is a blend of two high-biologic-value proteins: casein and soy.

| | | |
|----|-------------------------------|-----|
| | Sodium and calcium caseinates | 84% |
| 15 | Soy protein isolate | 16% |

Fat

The fat source is corn oil.

| | |
|----------|------|
| Corn oil | 100% |
|----------|------|

Carbohydrate

20 ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla

| | | |
|----|--------------|-----|
| 25 | Corn Syrup | 35% |
| | Maltodextrin | 35% |
| | Sucrose | 30% |

H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:

- For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

Features

- Rich and creamy, good taste
- Good source of essential vitamins and minerals Convenient-needs no refrigeration
- Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

Vanilla: ©-D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate. Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein

The protein source is nonfat milk.

Nonfat milk

100%

Fat

The fat source is hydrogenated soybean oil.

| | |
|--------------------------|------|
| Hydrogenated soybean oil | 100% |
|--------------------------|------|

Carbohydrate

- 5 ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors

| | | |
|----|----------------------|-----|
| 10 | Sucrose | 56% |
| | Lactose | 27% |
| | Modified food starch | 17% |

Chocolate

| | | |
|----|----------------------|-----|
| | Sucrose | 58% |
| 15 | Lactose | 26% |
| | Modified food starch | 16% |

I. ENSURE® WITH FIBER

- 20 Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is
- 25 suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions

- For patients who can benefit from increased dietary fiber and nutrients

Features

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- 5 • Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

Ingredients

- 10 **Vanilla:** ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride,
- 15 Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium
- 20 Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D₃ and Cyanocobalamin.

Protein

The protein source is a blend of two high-biologic-value proteins- casein and soy.

| | | |
|----|-------------------------------|-----|
| 25 | Sodium and calcium caseinates | 80% |
| | Soy protein isolate | 20% |

Fat

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

| | | |
|---|--------------------------|-----|
| | High-oleic safflower oil | 40% |
| 5 | Canola oil | 40% |
| | Corn oil | 20% |

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.

Carbohydrate

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors

| | | |
|----|--------------|-----|
| 20 | Maltodextrin | 66% |
| | Sucrose | 25% |
| | Oat Fiber | 7% |
| | Soy Fiber | 2% |

Chocolate

| | | |
|----|--------------|-----|
| 25 | Maltodextrin | 55% |
| | Sucrose | 36% |
| | Oat Fiber | 7% |

Soy Fiber

2%

Fiber

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl-oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs of this invention.

J. Oxepa™ Nutritional Product

Oxepa is low-carbohydrate, calorically dense enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric Distribution:

- Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs.
- The distribution of Calories in Oxepa is shown in Table 7.

| Table 7. Caloric Distribution of Oxepa | | | |
|--|--------------|-----------|----------|
| | per 8 fl oz. | per liter | % of Cal |
| Calories | 355 | 1,500 | --- |
| Fat (g) | 22.2 | 93.7 | 55.2 |
| Carbohydrate (g) | 25 | 105.5 | 28.1 |
| Protein (g) | 14.8 | 62.5 | 16.7 |
| Water (g) | 186 | 785 | --- |

Fat:

- Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).
- The fat source is a oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table 8.

- Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table 10.
- Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

5

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs of this invention.

Table 8. Typical Fatty Acid Profile

| | % Total Fatty Acids | g/8 fl oz* | g/L* |
|--------------------------------|---------------------|------------|-------|
| Caproic (6:0) | 0.2 | 0.04 | 0.18 |
| Caprylic (8:0) | 14.69 | 3.1 | 13.07 |
| Capric (10:0) | 11.06 | 2.33 | 9.87 |
| Palmitic (16:0) | 5.59 | 1.18 | 4.98 |
| Palmitoleic (16:1n-7) | 1.82 | 0.38 | 1.62 |
| Stearic (18:0) | 1.84 | 0.39 | 1.64 |
| Oleic (18:1n-9) | 24.44 | 5.16 | 21.75 |
| Linoleic (18:2n-6) | 16.28 | 3.44 | 14.49 |
| α -Linolenic (18:3n-3) | 3.47 | 0.73 | 3.09 |
| γ -Linolenic (18:3n-6) | 4.82 | 1.02 | 4.29 |
| Eicosapentaenoic (20:5n-3) | 5.11 | 1.08 | 4.55 |
| n-3-Docosapentaenoic (22:5n-3) | 0.55 | 0.12 | 0.49 |
| Docosahexaenoic (22:6n-3) | 2.27 | 0.48 | 2.02 |
| Others | 7.55 | 1.52 | 6.72 |

* Fatty acids equal approximately 95% of total fat.

Table 9. Fat Profile of Oxepa.

| | |
|------------------------------|------------------------------|
| % of total calories from fat | 55.2 |
| Polyunsaturated fatty acids | 31.44 g/L |
| Monounsaturated fatty acids | 25.53 g/L |
| Saturated fatty acids | 32.38 g/L |
| n-6 to n-3 ratio | 1.75:1 |
| Cholesterol | 9.49 mg/8 fl oz 40.1 mg/L |

10

Carbohydrate:

- The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).
- The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO₂) production. High CO₂ levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.
- Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

- Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).
- The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.
- Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO₂ production, a high protein diet will increase ventilatory drive.

- The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.
- As demonstrated in Table 11, the amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by theNational Academy of Sciences.
- Oxepa is gluten-free.

10 All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

15 The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

- 10 (i) APPLICANT: KNUTZON, DEBORAH
MURKERJI, PRADIP
HUANG, YUNG-SHENG
THURMOND, JENNIFER
CHAUDHARY, SUNITA
LEONARD, AMANDA
- 15 (ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR SYNTHESIS
OF LONG CHAIN POLY-UNSATURATED FATTY ACIDS IN PLANTS
- (iii) NUMBER OF SEQUENCES: 52
- 20 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LIMBACH & LIMBACH L.L.P.
(B) STREET: 2001 FERRY BUILDING
(C) CITY: SAN FRANCISCO
25 (D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94111
- (v) COMPUTER READABLE FORM:
30 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Microsoft Word
- 35 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- 40 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/834,033
(B) FILING DATE: 11-APR-1997
- (vii) PRIOR APPLICATION DATA:
45 (A) APPLICATION NUMBER: US 08/833,610
(B) FILING DATE: 11-APR-1997
- (viii) ATTORNEY/AGENT INFORMATION:
50 (A) NAME: MICHAEL R. WARD
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(C) REFERENCE/DOCKET NUMBER: CGAB-320
- (ix) TELECOMMUNICATION INFORMATION:
55 (A) TELEPHONE: (415) 433-4150
(B) TELEFAX: (415) 433-8716
(C) TELEX: N/A

(2) INFORMATION FOR SEQ ID NO:1:

- 60 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1617 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | |
|----|--|------|
| | CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCCTTC AACCCCCCTC TTTGACAAAG | 60 |
| | ACAACAAACC ATGGCTGCTG CTCCAGTGT GAGGACGTTT ACTCGGGCCG AGGTTTTGAA | 120 |
| 15 | TGCCGAGGCT CTGAATGAGG GCAAGAAGGA TGCCGAGGCA CCCTTCTTGA TGATCATCGA | 180 |
| | CAACAAGGTG TACGATGTCC GCGAGTTCGT CCCTGATCAT CCCGGTGGAA GTGTGATTCT | 240 |
| 20 | CACGCACGTT GGCAAGGACG GCACTGACGT CTTTGACACT TTTACCCCG AGGCTGCTTG | 300 |
| | GGAGACTCTT GCCAACTTTT ACGTTGGTGA TATTGACGAG AGCGACCGCG ATATCAAGAA | 360 |
| | TGATGACTTT GCGGCCGAGG TCCGCAAGCT GCGTACCTTG TTCCAGTCTC TTGGTTACTA | 420 |
| 25 | CGATTCTTCC AAGGCATACT ACGCCTTCAA GGTCTCGTTC AACCTCTGCA TCTGGGGTTT | 480 |
| | GTCGACGGTC ATTGTGGCCA AGTGGGGCCA GACCTCGACC CTCGCCAACG TGCTCTCGGC | 540 |
| 30 | TGCGCTTTTG GGTCTGTTCT GGCAGCAGTG CGGATGGTTG GCTCACGACT TTTTGCATCA | 600 |
| | CCAGGTCTTC CAGGACCGTT TCTGGGGTGA TCTTTTCGGC GCCTTCTTGG GAGGTGTCTG | 660 |
| | CCAGGGCTTC TCGTCCTCGT GGTGGAAGGA CAAGCACAAC ACTCACCACG CCGCCCCCAA | 720 |
| 35 | CGTCCACGGC GAGGATCCCG ACATTGACAC CCACCCTCTG TTGACCTGGA GTGAGCATGC | 780 |
| | GTTGGAGATG TTCTCGGATG TCCCAGATGA GGAGCTGACC CGCATGTGGT CGCGTTTCAT | 840 |
| 40 | GGTCCTGAAC CAGACCTGGT TTTACTTCCC CATTCTCTCG TTTGCCCCGC TCTCCTGGTG | 900 |
| | CCTCCAGTCC ATTCTCTTTG TGCTGCCTAA CGGTACAGGC CACAAGCCCT CGGGCGCGCG | 960 |
| | TGTGCCCATC TCGTTGGTCG AGCAGCTGTC GCTTGCGATG CACTGGACCT GGTACCTCGC | 1020 |
| 45 | CACCATGTTT CTGTTTCATCA AGGATCCCGT CAACATGCTG GTGTACTTTT TGGTGTGCGA | 1080 |
| | GGCGGTGTGC GGAAACTTGT TGGCGATCGT GTTCTCGCTC AACCACAACG GTATGCCTGT | 1140 |
| 50 | GATCTCGAAG GAGGAGGCGG TCGATATGGA TTTCTTCACG AAGCAGATCA TCACGGGTCG | 1200 |
| | TGATGTCCAC CCGGGTCTAT TTGCCAACTG GTTCACGGGT GGATTGAACT ATCAGATCGA | 1260 |
| | GCACCACTTG TTCCCTTCGA TGCCTCGCCA CAACTTTTCA AAGATCCAGC CTGCTGTCGA | 1320 |
| 55 | GACCCTGTGC AAAAAGTACA ATGTCCGATA CCACACCACC GGTATGATCG AGGGAAGTGC | 1380 |
| | AGAGGTCTTT AGCCGTCTGA ACGAGGTCTC CAAGGCTGCC TCCAAGATGG GTAAGGCGCA | 1440 |
| 60 | GTAAAAA AAAAAGGAC GTTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT | 1500 |
| | TGTCAGTTCG AGCGTTTCTG GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC | 1560 |

CCCCCGCTCA TATCTCATTC ATTTCTCTTA TTAAACAAC TGTTCACCG 1617

5

(2) INFORMATION FOR SEQ ID NO:2:

10

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 457 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25

Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu Val Leu
 1 5 10 15

30

Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala Pro Phe
 20 25 30

35

Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe Val Pro
 35 40 45

40

Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys Asp Gly
 50 55 60

45

Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu Thr Leu
 65 70 75 80

50

Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp Ile Lys
 85 90 95

55

Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu Phe Gln
 100 105 110

60

Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val
 115 120 125

Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val Ala Lys
 130 135 140

Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala Leu Leu
 145 150 155 160

Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe Leu His
 165 170 175

His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe
 180 185 190

Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys
 195 200 205

His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp Pro Asp
 210 215 220

Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met
 225 230 235 240
 5 Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg Phe
 245 250 255
 Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala
 260 265 270
 10 Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly
 275 280 285
 Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu
 290 295 300
 15 Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe
 305 310 315 320
 20 Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser
 325 330 335
 Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His
 340 345 350
 25 Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe
 355 360 365
 Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe
 370 375 380
 30 Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
 385 390 395 400
 35 Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val
 405 410 415
 Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met
 420 425 430
 40 Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys
 435 440 445
 Ala Ala Ser Lys Met Gly Lys Ala Gln
 450 455

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1488 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCCCTGTC GCTGTCGGCA CACCCCATCC TCCCTCGCTC CCTCTGCGTT TGTCCTTGGC 60

CCACCGTCTC TCCTCCACCC TCCGAGACGA CTGCAACTGT AATCAGGAAC CGACAAATAC 120
ACGATTTCTT TTTACTCAGC ACCAACTCAA AATCCTCAAC CGCAACCCTT TTTCAGGATG 180
5 GCACCTCCCA ACACTATCGA TGCCGGTTTG ACCCAGCGTC ATATCAGCAC CTCGGCCCCA 240
AACTCGGCCA AGCCTGCCTT CGAGCGCAAC TACCAGCTCC CCGAGTTCAC CATCAAGGAG 300
10 ATCCGAGAGT GCATCCCTGC CCACTGCTTT GAGCGCTCCG GTCTCCGTGG TCTCTGCCAC 360
GTTGCCATCG ATCTGACTTG GCGCTCGCTC TTGTTCTGG CTGCGACCCA GATCGACAAG 420
TTTGAGAATC CCTTGATCCG CTATTTGGCC TGGCCTGTTT ACTGGATCAT GCAGGGTATT 480
15 GTCTGCACCG GTGTCTGGGT GCTGGCTCAC GAGTGTGGTC ATCAGTCCTT CTCGACCTCC 540
AAGACCCTCA ACAACACAGT TGGTTGGATC TTGCACTCGA TGCTCTTGGT CCCCTACCAC 600
20 TCCTGGAGAA TCTCGCACTC GAAGCACCAC AAGGCCACTG GCCATATGAC CAAGGACCAG 660
GTCTTTGTGC CCAAGACCCG CTCCCAGGTT GGCTTGCCCTC CCAAGGAGAA CGCTGCTGCT 720
GCCGTTTCCAGG AGGAGGACAT GTCCGTGCAC CTGGATGAGG AGGCTCCCAT TGTGACTTTG 780
25 TTCTGGATGG TGATCCAGTT CTTGTTCCGA TGGCCCGCGT ACCTGATTAT GAACGCCTCT 840
GGCCAAGACT ACGGCCGCTG GACCTCGCAC TTCCACACGT ACTCGCCCAT CTTTGAGCCC 900
30 CGCAACTTTT TCGACATTAT TATCTCGGAC CTCGGTGTGT TGGCTGCCCT CGGTGCCCTG 960
ATCTATGCCT CCATGCAGTT GTCGCTCTTG ACCGTACCA AGTACTATAT TGTCCCCTAC 1020
CTCTTTGTCA ACTTTTGGTT GGTCTGATC ACCTTCTTGC AGCACACCGA TCCCAAGCTG 1080
35 CCCCATTACC GCGAGGGTGC CTGGAATTTT CAGCGTGGAG CTCTTTGCAC CGTTGACCGC 1140
TCGTTTGGCA AGTTCTTGGA CCATATGTTC CACGGCATTG TCCACACCCA TGTGGCCCAT 1200
40 CACTTGTTCT CGCAAATGCC GTTCTACCAT GCTGAGGAAG CTACCTATCA TCTCAAGAAA 1260
CTGCTGGGAG AGTACTATGT GTACGACCCA TCCCCGATCG TCGTTGCGGT CTGGAGGTCG 1320
TTCCGTGAGT GCCGATTCGT GGAGGATCAG GGAGACGTGG TCTTTTCAA GAAGTAAAAA 1380
45 AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC 1440
CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTCATTG GCGCCTCC 1488

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Met | Ala | Pro | Pro | Asn | Thr | Ile | Asp | Ala | Gly | Leu | Thr | Gln | Arg | His | Ile | |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| 5 | Ser | Thr | Ser | Ala | Pro | Asn | Ser | Ala | Lys | Pro | Ala | Phe | Glu | Arg | Asn | Tyr | |
| | | | | 20 | | | | | 25 | | | | | 30 | | | |
| | Gln | Leu | Pro | Glu | Phe | Thr | Ile | Lys | Glu | Ile | Arg | Glu | Cys | Ile | Pro | Ala | |
| 10 | | | 35 | | | | | 40 | | | | | 45 | | | | |
| | His | Cys | Phe | Glu | Arg | Ser | Gly | Leu | Arg | Gly | Leu | Cys | His | Val | Ala | Ile | |
| | | 50 | | | | | 55 | | | | | 60 | | | | | |
| 15 | Asp | Leu | Thr | Trp | Ala | Ser | Leu | Leu | Phe | Leu | Ala | Ala | Thr | Gln | Ile | Asp | |
| | 65 | | | | | 70 | | | | | 75 | | | | | 80 | |
| | Lys | Phe | Glu | Asn | Pro | Leu | Ile | Arg | Tyr | Leu | Ala | Trp | Pro | Val | Tyr | Trp | |
| | | | | | 85 | | | | | 90 | | | | | 95 | | |
| 20 | Ile | Met | Gln | Gly | Ile | Val | Cys | Thr | Gly | Val | Trp | Val | Leu | Ala | His | Glu | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | |
| | Cys | Gly | His | Gln | Ser | Phe | Ser | Thr | Ser | Lys | Thr | Leu | Asn | Asn | Thr | Val | |
| 25 | | | 115 | | | | | 120 | | | | | 125 | | | | |
| | Gly | Trp | Ile | Leu | His | Ser | Met | Leu | Leu | Val | Pro | Tyr | His | Ser | Trp | Arg | |
| | | 130 | | | | | 135 | | | | | 140 | | | | | |
| 30 | Ile | Ser | His | Ser | Lys | His | His | Lys | Ala | Thr | Gly | His | Met | Thr | Lys | Asp | |
| | 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| | Gln | Val | Phe | Val | Pro | Lys | Thr | Arg | Ser | Gln | Val | Gly | Leu | Pro | Pro | Lys | |
| | | | | | 165 | | | | | 170 | | | | | 175 | | |
| 35 | Glu | Asn | Ala | Ala | Ala | Ala | Val | Gln | Glu | Glu | Asp | Met | Ser | Val | His | Leu | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| | Asp | Glu | Glu | Ala | Pro | Ile | Val | Thr | Leu | Phe | Trp | Met | Val | Ile | Gln | Phe | |
| 40 | | | 195 | | | | | 200 | | | | | 205 | | | | |
| | Leu | Phe | Gly | Trp | Pro | Ala | Tyr | Leu | Ile | Met | Asn | Ala | Ser | Gly | Gln | Asp | |
| | | 210 | | | | | 215 | | | | | 220 | | | | | |
| 45 | Tyr | Gly | Arg | Trp | Thr | Ser | His | Phe | His | Thr | Tyr | Ser | Pro | Ile | Phe | Glu | |
| | 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| | Pro | Arg | Asn | Phe | Phe | Asp | Ile | Ile | Ile | Ser | Asp | Leu | Gly | Val | Leu | Ala | |
| | | | | | 245 | | | | | 250 | | | | | 255 | | |
| 50 | Ala | Leu | Gly | Ala | Leu | Ile | Tyr | Ala | Ser | Met | Gln | Leu | Ser | Leu | Leu | Thr | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| | Val | Thr | Lys | Tyr | Tyr | Ile | Val | Pro | Tyr | Leu | Phe | Val | Asn | Phe | Trp | Leu | |
| 55 | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Val | Leu | Ile | Thr | Phe | Leu | Gln | His | Thr | Asp | Pro | Lys | Leu | Pro | His | Tyr | |
| | | 290 | | | | | 295 | | | | | 300 | | | | | |
| 60 | Arg | Glu | Gly | Ala | Trp | Asn | Phe | Gln | Arg | Gly | Ala | Leu | Cys | Thr | Val | Asp | |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 | |

Arg Ser Phe Gly Lys Phe Leu Asp His Met Phe His Gly Ile Val His
 325 330 335
 5 Thr His Val Ala His His Leu Phe Ser Gln Met Pro Phe Tyr His Ala
 340 345 350
 Glu Glu Ala Thr Tyr His Leu Lys Lys Leu Leu Gly Glu Tyr Tyr Val
 355 360 365
 10 Tyr Asp Pro Ser Pro Ile Val Val Ala Val Trp Arg Ser Phe Arg Glu
 370 375 380
 Cys Arg Phe Val Glu Asp Gln Gly Asp Val Val Phe Phe Lys Lys
 385 390 395
 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1483 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 20

(ii) MOLECULE TYPE: DNA (genomic)
 25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 GCTTCCTCCA GTTCATCCTC CATTTCGCCA CCTGCATTCT TTACGACCGT TAAGCAAGAT 60
 GGGAACGGAC CAAGGAAAAA CCTTCACCTG GGAAGAGCTG GCGGCCCATTA ACACCAAGGA 120
 35 CGACCTACTC TTGGCCATCC GCGGCAGGGT GTACGATGTC ACAAAGTTCT TGAGCCGCCA 180
 TCCTGGTGGA GTGGACACTC TCCTGCTCGG AGCTGGCCGA GATGTTACTC CGGTCTTTGA 240
 40 GATGTATCAC GCGTTTGGGG CTGCAGATGC CATTATGAAG AAGTACTATG TCGGTACACT 300
 GGTCTCGAAT GAGCTGCCCA TCTTCCCGGA GCCAACGGTG TTCCACAAAA CCATCAAGAC 360
 GAGAGTCGAG GGCTACTTTA CGGATCGGAA CATTGATCCC AAGAATAGAC CAGAGATCTG 420
 45 GGGACGATAC GCTCTTATCT TTGGATCCTT GATCGCTTCC TACTACGCGC AGCTCTTTGT 480
 GCCTTTCGTT GTCGAACGCA CATGGCTTCA GGTGGTGTTT GCAATCATCA TGGGATTTGC 540
 50 GTGCGCACAA GTCGGACTCA ACCCTCTTCA TGATGCGTCT CACTTTTCAG TGACCCACAA 600
 CCCCACGTG TCGAAGATTC TGGGAGCCAC GCACGACTTT TTCAACGGAG CATCGTACCT 660
 GGTGTGGATG TACCAACATA TGCTCGGCCA TCACCCCTAC ACCAACATTG CTGGAGCAGA 720
 55 TCCCGACGTG TCGACGTCTG AGCCCGATGT TCGTCGTATC AAGCCCAACC AAAAGTGTTT 780
 TGTCAACCAC ATCAACCAGC ACATGTTTGT TCCTTTCCTG TACGGACTGC TGGCGTTCAA 840
 60 GGTGCGCATT CAGGACATCA ACATTTTGTA CTTTGTCAAG ACCAATGACG CTATTCGTGT 900
 CAATCCCATC TCGACATGGC AACTGTGAT GTTCTGGGGC GGCAAGGCTT TCTTTGTCTG 960

GTATCGCCTG ATTGTTCCCC TGCAGTATCT GCCCCTGGGC AAGGTGCTGC TCTTGTTTAC 1020
 GGTGCGGGAC ATGGTGTCGT CTTACTGGCT GGCGCTGACC TTCCAGGCGA ACCACGTTGT 1080
 5 TGAGGAAGTT CAGTGGCCGT TGCCTGACGA GAACGGGATC ATCCAAAAGG ACTGGGCAGC 1140
 TATGCAGGTC GAGACTACGC AGGATTACGC ACACGATTCTG CACCTCTGGA CCAGCATCAC 1200
 10 TGGCAGCTTG AACTACCAGG CTGTGCACCA TCTGTTCCCC AACGTGTCGC AGCACCATTA 1260
 TCCCGATATT CTGGCCATCA TCAAGAACAC CTGCAGCGAG TACAAGGTTT CATACTTGT 1320
 CAAGGATACG TTTTGGCAAG CATTGCTTC ACATTTGGAG CACTTGCGTG TTCTTGGACT 1380
 15 CCGTCCCAAG GAAGAGTAGA AGAAAAAAG CGCCGAATGA AGTATTGCCC CCTTTTCTC 1440
 CAAGAATGGC AAAAGGAGAT CAAGTGGACA TTCTCTATGA AGA 1483

20 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 446 amino acids
 (B) TYPE: amino acid
 25 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35 Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala
 1 5 10 15
 His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr
 20 25 30
 40 Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu
 35 40 45
 Leu Leu Gly Ala Gly Arg Asp Val Thr Pro Val Phe Glu Met Tyr His
 50 55 60
 Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr
 65 70 75 80
 50 Leu Val Ser Asn Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His
 85 90 95
 Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile
 100 105 110
 55 Asp Pro Lys Asn Arg Pro Glu Ile Trp Gly Arg Tyr Ala Leu Ile Phe
 115 120 125
 Gly Ser Leu Ile Ala Ser Tyr Tyr Ala Gln Leu Phe Val Pro Phe Val
 130 135 140
 60 Val Glu Arg Thr Trp Leu Gln Val Val Phe Ala Ile Ile Met Gly Phe
 145 150 155 160

Ala Cys Ala Gln Val Gly Leu Asn Pro Leu His Asp Ala Ser His Phe
 165 170 175
 5 Ser Val Thr His Asn Pro Thr Val Trp Lys Ile Leu Gly Ala Thr His
 180 185 190
 Asp Phe Phe Asn Gly Ala Ser Tyr Leu Val Trp Met Tyr Gln His Met
 195 200 205
 10 Leu Gly His His Pro Tyr Thr Asn Ile Ala Gly Ala Asp Pro Asp Val
 210 215 220
 Ser Thr Ser Glu Pro Asp Val Arg Arg Ile Lys Pro Asn Gln Lys Trp
 225 230 235 240
 15 Phe Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly
 245 250 255
 20 Leu Leu Ala Phe Lys Val Arg Ile Gln Asp Ile Asn Ile Leu Tyr Phe
 260 265 270
 Val Lys Thr Asn Asp Ala Ile Arg Val Asn Pro Ile Ser Thr Trp His
 275 280 285
 25 Thr Val Met Phe Trp Gly Gly Lys Ala Phe Phe Val Trp Tyr Arg Leu
 290 295 300
 30 Ile Val Pro Leu Gln Tyr Leu Pro Leu Gly Lys Val Leu Leu Leu Phe
 305 310 315 320
 Thr Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln
 325 330 335
 35 Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn
 340 345 350
 Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln
 355 360 365
 40 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu
 370 375 380
 Asn Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His
 385 390 395 400
 45 Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys
 405 410 415
 50 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His
 420 425 430
 Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu
 435 440 445
 55

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 355 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10

Glu Val Arg Lys Leu Arg Thr Leu Phe Gln Ser Leu Gly Tyr Tyr Asp
1 5 10 15

Ser Ser Lys Ala Tyr Tyr Ala Phe Lys Val Ser Phe Asn Leu Cys Ile
20 25 30

15

Trp Gly Leu Ser Thr Val Ile Val Ala Lys Trp Gly Gln Thr Ser Thr
35 40 45

20

Leu Ala Asn Val Leu Ser Ala Ala Leu Leu Gly Leu Phe Trp Gln Gln
50 55 60

Cys Gly Trp Leu Ala His Asp Phe Leu His His Gln Val Phe Gln Asp
65 70 75 80

25

Arg Phe Trp Gly Asp Leu Phe Gly Ala Phe Leu Gly Gly Val Cys Gln
85 90 95

Gly Phe Ser Ser Ser Trp Trp Lys Asp Lys His Asn Thr His His Ala
100 105 110

30

Ala Pro Asn Val His Gly Glu Asp Pro Asp Ile Asp Thr His Pro Leu
115 120 125

Leu Thr Trp Ser Glu His Ala Leu Glu Met Phe Ser Asp Val Pro Asp
130 135 140

35

Glu Glu Leu Thr Arg Met Trp Ser Arg Phe Met Val Leu Asn Gln Thr
145 150 155 160

40

Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala Arg Leu Ser Trp Cys Leu
165 170 175

Gln Ser Ile Leu Phe Val Leu Pro Asn Gly Gln Ala His Lys Pro Ser
180 185 190

45

Gly Ala Arg Val Pro Ile Ser Leu Val Glu Gln Leu Ser Leu Ala Met
195 200 205

His Trp Thr Trp Tyr Leu Ala Thr Met Phe Leu Phe Ile Lys Asp Pro
210 215 220

50

Val Asn Met Leu Val Tyr Phe Leu Val Ser Gln Ala Val Cys Gly Asn
225 230 235 240

Leu Leu Ala Ile Val Phe Ser Leu Asn His Asn Gly Met Pro Val Ile
245 250 255

55

Ser Lys Glu Glu Ala Val Asp Met Asp Phe Phe Thr Lys Gln Ile Ile
260 265 270

60

Thr Gly Arg Asp Val His Pro Gly Leu Phe Ala Asn Trp Phe Thr Gly
275 280 285

Gly Leu Asn Tyr Gln Ile Glu His His Leu Phe Pro Ser Met Pro Arg
 290 295 300
 5 His Asn Phe Ser Lys Ile Gln Pro Ala Val Glu Thr Leu Cys Lys Lys
 305 310 315 320
 Tyr Asn Val Arg Tyr His Thr Thr Gly Met Ile Glu Gly Thr Ala Glu
 325 330 335
 10 Val Phe Ser Arg Leu Asn Glu Val Ser Lys Ala Ala Ser Lys Met Gly
 340 345 350
 Lys Ala Gln
 15 355

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 104 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Thr Leu Tyr Thr Leu Ala Phe Val Ala Ala Asn Ser Leu Gly Val
 1 5 10 15
 35 Leu Tyr Gly Val Leu Ala Cys Pro Ser Val Xaa Pro His Gln Ile Ala
 20 25 30
 Ala Gly Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile Gly Xaa
 35 40 45
 40 Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Asn Asn Xaa Phe
 50 55 60
 45 Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ile Ala Trp Trp
 65 70 75 80
 Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser Leu Asp Tyr
 85 90 95
 50 Gly Pro Asn Leu Gln His Ile Pro
 100

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 252 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

5 Gly Val Leu Tyr Gly Val Leu Ala Cys Thr Ser Val Phe Ala His Gln
 1 5 10 15
 10 Ile Ala Ala Ala Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile
 20 25 30
 Gly His Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Tyr Asn
 35 40 45
 15 Arg Phe Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ser Ile
 50 55 60
 Ala Trp Trp Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser
 65 70 75 80
 20 Leu Asp Tyr Asp Pro Asp Leu Gln His Ile Pro Val Phe Ala Val Ser
 85 90 95
 Thr Lys Phe Phe Ser Ser Leu Thr Ser Arg Phe Tyr Asp Arg Lys Leu
 100 105 110
 25 Thr Phe Gly Pro Val Ala Arg Phe Leu Val Ser Tyr Gln His Phe Thr
 115 120 125
 30 Tyr Tyr Pro Val Asn Cys Phe Gly Arg Ile Asn Leu Phe Ile Gln Thr
 130 135 140
 Phe Leu Leu Leu Phe Ser Lys Arg Glu Val Pro Asp Arg Ala Leu Asn
 145 150 155 160
 35 Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro Leu Leu Val Ser
 165 170 175
 Cys Leu Pro Asn Trp Pro Glu Arg Phe Phe Phe Val Phe Thr Ser Phe
 180 185 190
 40 Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu Asn His Phe Ala
 195 200 205
 45 Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp Trp Phe Glu Lys
 210 215 220
 Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser Tyr Met Asp Trp
 225 230 235 240
 50 Phe Phe Gly Gly Leu Gln Phe Gln Leu Glu His His
 245 250

(2) INFORMATION FOR SEQ ID NO:10:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear
 60 (ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Xaa Xaa Asn Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro
 1 5 10 15

10 Leu Leu Val Ser Cys Leu Pro Asn Trp Pro Glu Arg Phe Xaa Phe Val
 20 25 30

Phe Thr Gly Phe Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu
 35 40 45

15 Asn His Phe Ala Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp
 50 55 60

Trp Phe Glu Lys Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser
 65 70 75 80

20 Tyr Met Asp Trp Phe Phe Cys Gly Leu Gln Phe Gln Leu Glu His His
 85 90 95

25 Leu Phe Pro Arg Leu Pro Arg Cys His Leu Arg Lys Val Ser Pro Val
 100 105 110

Gly Gln Arg Gly Phe Gln Arg Lys Xaa Asn Leu Ser Xaa
 115 120 125

30 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

35 (B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Ala Thr Glu Val Gly Gly Leu Ala Trp Met Ile Thr Phe Tyr Val
 1 5 10 15

Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys Ala Phe Leu
 20 25 30

50 Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp Phe Val Trp
 35 40 45

55 Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His Asp Arg Asn
 50 55 60

Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn Val His Lys
 65 70 75 80

60 Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile Glu
 85 90 95

His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His Xaa Val Ala
 100 105 110
 5 Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser
 115 120 125
 Lys Pro Leu
 130

10 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 87 amino acids
 15 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Ser Pro Lys Ser Ser Pro Thr Arg Asn Met Thr Pro Ser Pro Phe
 1 5 10 15
 Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu
 20 25 30
 Phe Pro Thr Met Pro Arg Cys Asn Leu Asn Arg Cys Met Lys Tyr Val
 35 40 45
 Lys Glu Trp Cys Ala Glu Asn Asn Leu Pro Tyr Leu Val Asp Asp Tyr
 50 55 60
 Phe Val Gly Tyr Asn Leu Asn Leu Gln Gln Leu Lys Asn Met Ala Glu
 65 70 75 80
 40 Leu Val Gln Ala Lys Ala Ala
 85

(2) INFORMATION FOR SEQ ID NO:13:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 143 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg His Glu Ala Ala Arg Gly Gly Thr Arg Leu Ala Tyr Met Leu Val
 1 5 10 15
 60 Cys Met Gln Trp Thr Asp Leu Leu Trp Ala Ala Ser Phe Tyr Ser Arg
 20 25 30

| | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Phe | Phe | Leu | Ser | Tyr | Ser | Pro | Phe | Tyr | Gly | Ala | Thr | Gly | Thr | Leu | Leu |
| | | | 35 | | | | | 40 | | | | | 45 | | | |
| 5 | Leu | Phe | Val | Ala | Val | Arg | Val | Leu | Glu | Ser | His | Trp | Phe | Val | Trp | Ile |
| | | 50 | | | | | 55 | | | | | 60 | | | | |
| | Thr | Gln | Met | Asn | His | Ile | Pro | Lys | Glu | Ile | Gly | His | Glu | Lys | His | Arg |
| 10 | 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| | Asp | Trp | Ala | Ser | Ser | Gln | Leu | Ala | Ala | Thr | Cys | Asn | Val | Glu | Pro | Ser |
| | | | | 85 | | | | | | 90 | | | | | 95 | |
| | Leu | Phe | Ile | Asp | Trp | Phe | Ser | Gly | His | Leu | Asn | Phe | Gln | Ile | Glu | His |
| 15 | | | | 100 | | | | | 105 | | | | | 110 | | |
| | His | Leu | Phe | Pro | Thr | Met | Thr | Arg | His | Asn | Tyr | Arg | Xaa | Val | Ala | Pro |
| | | | 115 | | | | | 120 | | | | | 125 | | | |
| | Leu | Val | Lys | Ala | Phe | Cys | Ala | Lys | His | Gly | Leu | His | Tyr | Glu | Val | |
| 20 | | 130 | | | | | 135 | | | | | 140 | | | | |

(2) INFORMATION FOR SEQ ID NO:14:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 186 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
26 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

| | | | | | | | | | | | | | | | | |
|----|-----------|-----------|------------|------------|-----------|-----------|-----|------------|------------|-----------|-----------|-----------|------------|-----------|-----------|-----------|
| 40 | Leu 1 | His | His | Thr | Tyr 5 | Thr | Asn | Ile | Ala | Gly 10 | Ala | Asp | Pro | Asp | Val | Ser |
| | Thr | Ser | Glu | Pro 20 | Asp | Val | Arg | Arg | Ile 25 | Lys | Pro | Asn | Gln | Lys 30 | Trp | Phe |
| 45 | Val | Asn | His 35 | Ile | Asn | Gln | His | Met 40 | Phe | Val | Pro | Phe | Leu 45 | Tyr | Gly | Leu |
| | Leu | Ala 50 | Phe | Lys | Val | Arg 55 | Ile | Gln | Asp | Ile | Asn | Ile 60 | Leu | Tyr | Phe | Val |
| 50 | Lys 65 | Thr | Asn | Asp | Ala | Ile 70 | Arg | Val | Asn | Pro | Ile 75 | Ser | Thr | Trp | His | Thr 80 |
| | Val | Met | Phe | Trp | Gly 85 | Gly | Lys | Ala | Phe | Phe 90 | Val | Trp | Tyr | Arg | Leu 95 | Ile |
| 55 | Val | Pro | Leu | Gln 100 | Tyr | Leu | Pro | Leu | Gly 105 | Lys | Val | Leu | Leu | Leu | Phe | Thr |
| 60 | Val | Ala | Asp 115 | Met | Val | Ser | Ser | Tyr 120 | Trp | Leu | Ala | Leu | Thr 125 | Phe | Gln | Ala |

Asn Tyr Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn Gly
 130 135 140
 5 Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln Asp
 145 150 155 160
 Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu Asn
 165 170 175
 10 Tyr Gln Xaa Val His His Leu Phe Pro His
 180 185

(2) INFORMATION FOR SEQ ID NO:15:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Xaa Xaa His His
 1 5

30

(2) INFORMATION FOR SEQ ID NO:16:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 446 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn
 1 5 10 15

50

His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr
 20 25 30

Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu
 35 40 45

55

Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
 50 55 60

60

Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr
 65 70 75 80

Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Val Tyr Arg Lys Leu
 85 90 95

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | Val | Phe | Glu | Phe | Ser | Lys | Met | Gly | Leu | Tyr | Asp | Lys | Lys | Gly | His | Ile | |
| | | | | 100 | | | | | 105 | | | | | 110 | | | |
| 5 | Met | Phe | Ala | Thr | Leu | Cys | Phe | Ile | Ala | Met | Leu | Phe | Ala | Met | Ser | Val | |
| | | | 115 | | | | | 120 | | | | | 125 | | | | |
| | Tyr | Gly | Val | Leu | Phe | Cys | Glu | Gly | Val | Leu | Val | His | Leu | Phe | Ser | Gly | |
| 10 | | 130 | | | | | 135 | | | | | 140 | | | | | |
| | Cys | Leu | Met | Gly | Phe | Leu | Trp | Ile | Gln | Ser | Gly | Trp | Ile | Gly | His | Asp | |
| | 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| | Ala | Gly | His | Tyr | Met | Val | Val | Ser | Asp | Ser | Arg | Leu | Asn | Lys | Phe | Met | |
| 15 | | | | | 165 | | | | | 170 | | | | | 175 | | |
| | Gly | Ile | Phe | Ala | Ala | Asn | Cys | Leu | Ser | Gly | Ile | Ser | Ile | Gly | Trp | Trp | |
| | | | | 180 | | | | | 185 | | | | | 190 | | | |
| 20 | Lys | Trp | Asn | His | Asn | Ala | His | His | Ile | Ala | Cys | Asn | Ser | Leu | Glu | Tyr | |
| | | | 195 | | | | | 200 | | | | | 205 | | | | |
| | Asp | Pro | Asp | Leu | Gln | Tyr | Ile | Pro | Phe | Leu | Val | Val | Ser | Ser | Lys | Phe | |
| 25 | | 210 | | | | | 215 | | | | | 220 | | | | | |
| | Phe | Gly | Ser | Leu | Thr | Ser | His | Phe | Tyr | Glu | Lys | Arg | Leu | Thr | Phe | Asp | |
| | 225 | | | | | 230 | | | | 235 | | | | | | 240 | |
| | Ser | Leu | Ser | Arg | Phe | Phe | Val | Ser | Tyr | Gln | His | Trp | Thr | Phe | Tyr | Pro | |
| 30 | | | | | 245 | | | | | 250 | | | | | 255 | | |
| | Ile | Met | Cys | Ala | Ala | Arg | Leu | Asn | Met | Tyr | Val | Gln | Ser | Leu | Ile | Met | |
| | | | | 260 | | | | | 265 | | | | | 270 | | | |
| 35 | Leu | Leu | Thr | Lys | Arg | Asn | Val | Ser | Tyr | Arg | Ala | Gln | Glu | Leu | Leu | Gly | |
| | | | 275 | | | | | 280 | | | | | 285 | | | | |
| | Cys | Leu | Val | Phe | Ser | Ile | Trp | Tyr | Pro | Leu | Leu | Val | Ser | Cys | Leu | Pro | |
| 40 | | 290 | | | | | 295 | | | | | 300 | | | | | |
| | Asn | Trp | Gly | Glu | Arg | Ile | Met | Phe | Val | Ile | Ala | Ser | Leu | Ser | Val | Thr | |
| | 305 | | | | | 310 | | | | | 315 | | | | | 320 | |
| | Gly | Met | Gln | Gln | Val | Gln | Phe | Ser | Leu | Asn | His | Phe | Ser | Ser | Ser | Val | |
| 45 | | | | | 325 | | | | | 330 | | | | | 335 | | |
| | Tyr | Val | Gly | Lys | Pro | Lys | Gly | Asn | Asn | Trp | Phe | Glu | Lys | Gln | Thr | Asp | |
| | | | | 340 | | | | 345 | | | | | | 350 | | | |
| 50 | Gly | Thr | Leu | Asp | Ile | Ser | Cys | Pro | Pro | Trp | Met | Asp | Trp | Phe | His | Gly | |
| | | | 355 | | | | | 360 | | | | | 365 | | | | |
| | Gly | Leu | Gln | Phe | Gln | Ile | Glu | His | His | Leu | Phe | Pro | Lys | Met | Pro | Arg | |
| 55 | | 370 | | | | | 375 | | | | | 380 | | | | | |
| | Cys | Asn | Leu | Arg | Lys | Ile | Ser | Pro | Tyr | Val | Ile | Glu | Leu | Cys | Lys | Lys | |
| | 385 | | | | | 390 | | | | | 395 | | | | | 400 | |
| | His | Asn | Leu | Pro | Tyr | Asn | Tyr | Ala | Ser | Phe | Ser | Lys | Ala | Asn | Glu | Met | |
| 60 | | | | | 405 | | | | | 410 | | | | | 415 | | |

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
 420 425 430

5 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr
 435 440 445

(2) INFORMATION FOR SEQ ID NO:17:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 359 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
 1 5 10 15
 25 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
 20 25 30
 30 Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
 35 40 45
 Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
 50 55 60
 35 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
 65 70 75 80
 Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
 85 90 95
 40 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
 100 105 110
 45 Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
 115 120 125
 Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
 130 135 140
 50 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
 145 150 155 160
 Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
 165 170 175
 55 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
 180 185 190
 His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
 195 200 205
 60 Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
 210 215 220

5 Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
 225 230 235 240
 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
 245 250 255
 10 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
 260 265 270
 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
 275 280 285
 15 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
 290 295 300
 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
 305 310 315 320
 20 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
 325 330 335
 Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
 340 345 350
 25 Glu Ala Met Gly Lys Ala Ser
 355

(2) INFORMATION FOR SEQ ID NO:18:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 365 amino acids
 (B) TYPE: amino acid
 35 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

45 Met Thr Ser Thr Thr Ser Lys Val Thr Phe Gly Lys Ser Ile Gly Phe
 1 5 10 15
 Arg Lys Glu Leu Asn Arg Arg Val Asn Ala Tyr Leu Glu Ala Glu Asn
 20 25 30
 50 Ile Ser Pro Arg Asp Asn Pro Pro Met Tyr Leu Lys Thr Ala Ile Ile
 35 40 45
 Leu Ala Trp Val Val Ser Ala Trp Thr Phe Val Val Phe Gly Pro Asp
 50 55 60
 55 Val Leu Trp Met Lys Leu Leu Gly Cys Ile Val Leu Gly Phe Gly Val
 65 70 75 80
 60 Ser Ala Val Gly Ph Asn Ile Ser His Asp Gly Asn His Gly Gly Tyr
 85 90 95

5 Ser Lys Tyr Gln Trp Val Asn Tyr Leu Ser Gly Leu Thr His Asp Ala
 100 105 110
 Ile Gly Val Ser Ser Tyr Leu Trp Lys Phe Arg His Asn Val Leu His
 115 120 125
 His Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp
 130 135 140
 10 Glu Leu Val Arg Met Ser Pro Ser Met Glu Tyr Arg Trp Tyr His Arg
 145 150 155 160
 Tyr Gln His Trp Phe Ile Trp Phe Val Tyr Pro Phe Ile Pro Tyr Tyr
 165 170 175
 15 Trp Ser Ile Ala Asp Val Gln Thr Met Leu Phe Lys Arg Gln Tyr His
 180 185 190
 20 Asp His Glu Ile Pro Ser Pro Thr Trp Val Asp Ile Ala Thr Leu Leu
 195 200 205
 Ala Phe Lys Ala Phe Gly Val Ala Val Phe Leu Ile Ile Pro Ile Ala
 210 215 220
 25 Val Gly Tyr Ser Pro Leu Glu Ala Val Ile Gly Ala Ser Ile Val Tyr
 225 230 235 240
 Met Thr His Gly Leu Val Ala Cys Val Val Phe Met Leu Ala His Val
 245 250 255
 30 Ile Glu Pro Ala Glu Phe Leu Asp Pro Asp Asn Leu His Ile Asp Asp
 260 265 270
 35 Glu Trp Ala Ile Ala Gln Val Lys Thr Thr Val Asp Phe Ala Pro Asn
 275 280 285
 Asn Thr Ile Ile Asn Trp Tyr Val Gly Gly Leu Asn Tyr Gln Thr Val
 290 295 300
 40 His His Leu Phe Pro His Ile Cys His Ile His Tyr Pro Lys Ile Ala
 305 310 315 320
 Pro Ile Leu Ala Glu Val Cys Glu Glu Phe Gly Val Asn Tyr Ala Val
 325 330 335
 45 His Gln Thr Phe Phe Gly Ala Leu Ala Ala Asn Tyr Ser Trp Leu Lys
 340 345 350
 50 Lys Met Ser Ile Asn Pro Glu Thr Lys Ala Ile Glu Gln
 355 360 365

(2) INFORMATION FOR SEQ ID NO:19:

- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 35 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 60 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 CCAAGCTTCT GCAGGAGCTC TTTTTTTTTT TTTT 35

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

20 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 21
(D) OTHER INFORMATION: /number= 1
/note= "N=Inosine or Cytosine"

25 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 27
(D) OTHER INFORMATION: /number= 2
/note= "N=Inosine or Cytosine"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 CUACUACUAC UACAYCAYAC NTAYACNAAY AT 32

(2) INFORMATION FOR SEQ ID NO:21:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

50 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 13
(D) OTHER INFORMATION: /number= 1
/note= "N=Inosine or Cytosine"

55 (ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 19
(D) OTHER INFORMATION: /number= 2
/note= "N=Inosine or Cytosine"

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAUCAUCAUC AUNGGRAANA RRTGRTG 27

(2) INFORMATION FOR SEQ ID NO:22:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CUACUACUAC UAGGAGTCCT CTACGGTGTT TTG 33

20 (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

35 CAUCAUCAUC AUATGATGCT CAAGCTGAAA CTG 33

(2) INFORMATION FOR SEQ ID NO:24:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Xaa Xaa His His
1 5

55 (2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
60 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CUACUACUAC UACTCGAGCA AGATGGGAAC GGACCAAGG

39

10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

25

CAUCAUCAUC AUCTCGAGCT ACTCTTCCTT GGGACGGAG

39

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CUACUACUAC UATCTAGACT CGAGACCATG GCTGCTGCTC CAGTGTG

47

(2) INFORMATION FOR SEQ ID NO:28:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

50

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CAUCAUCAUC AUAGGCCTCG AGTTACTGCG CCTTACCCAT

60

40

(2) INFORMATION FOR SEQ ID NO:29:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

15 CUACUACUA CUAGGATCCA TGGCACCTCC CAACACT 37

(2) INFORMATION FOR SEQ ID NO:30:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 42 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

30 CAUCAUCAU CAUGGTACCT CGAGTTACTT CTTGAAAAAG AC 42

(2) INFORMATION FOR SEQ ID NO:31:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1219 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2692004)
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCACGCCGAC CGGCGCCGGG AGATCCTGGC AAAGTATCCA GAGATAAAGT CCTTGATGAA 60

50 ACCTGATCCC AATTGATAT GGATTATAAT TATGATGGTT CTCACCCAGT TGGGTGCATT 120

TTACATAGTA AAAGACTTGG ACTGGAAATG GGTCATATTT GGGGCCTATG CGTTTGGCAG 180

55 TTGCATTAAC CACTCAATGA CTCTGGCTAT TCATGAGATT GCCACAATG CTGCCTTTGG 240

CAACTGCAAA GCAATGTGGA ATCGCTGGTT TGGAAATGTTT GCTAATCTTC CTATTGGGAT 300

TCCATATTCA ATTTCTTTA AGAGGTATCA CATGGATCAT CATCGGTACC TTGGAGCTGA 360

60 TGGCGTCGAT GTAGATATTC CTACCGATTT TGAGGGCTGG TTCTTCTGTA CCGCTTTCAG 420

AAAGTTTATA TGGGTTATTC TTCAGCCTCT CTTTATGCC TTTCGACCTC TGTTTCATCAA 480

5 CCCCCAACCA ATTACGTATC TGGAAGTTAT CAATACCGTG GCACAGGTCA CTTTGTGACAT 540
 TTTAATTTAT TACTTTTTTGG GAATTAAATC CTTAGTCTAC ATGTTGGCAG CATCTTTACT 600
 TGGCCTGGGT TTGCACCCAA TTTCTGGACA TTTTATAGCT GAGCATTACA TGTCTTAAA 660
 GGGTCATGAA ACTTACTCAT ATTATGGGCC TCTGAATTTA CTTACCTTCA ATGTGGGTTA 720
 10 TCATAATGAA CATCATGATT TCCCCAACAT TCCTGGAAAA AGTCTTCCAC TGGTGAGGAA 780
 AATAGCAGCT GAATACTATG ACAACCTCCC TCACTACAAT TCCTGGATAA AAGTACTGTA 840
 15 TGATTTTGTG ATGGATGATA CAATAAGTCC CTACTIONAAGA ATGAAGAGGC ACCAAAAAGG 900
 AGAGATGGTG CTGGAGTAAA TATCATTAGT GCCAAAGGGA TTCTTCTCCA AAACCTTAGA 960
 TGATAAAATG GAATTTTTTGC ATTATTAAAC TTGAGACCAG TGATGCTCAG AAGCTCCCCT 1020
 20 GGCACAATTT CAGAGTAAGA GCTCGGTGAT ACCAAGAAGT GAATCTGGCT TTTAAACAGT 1080
 CAGCCTGACT CTGTACTGCT CAGTTTCACT CACAGGAAAC TTGTGACTTG TGTATTATCG 1140
 25 TCATTGAGGA TGTTTCACTC ATGTCTGTCA TTTTATAAGC ATATCATTTA AAAAGCTTCT 1200
 AAAAAGCTAT TTCGCCAGG 1219

30 (2) INFORMATION FOR SEQ ID NO:32:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 655 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2153526)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

45 TTACCTTCTA CGTCCGCTTC TTCCTCACTT ATGTGCCACT ATTGGGGCTG AAAGCTTCCT 60
 GGGCCTTTTC TTCATAGTCA GGTTCCTGGA AAGCAACTGG TTTGTGTGGG TGACACAGAT 120
 GAACCATATT CCCATGCACA TTGATCATGA CCGGAACATG GACTGGGTTT CCACCCAGCT 180
 50 CCAGGCCACA TGCAATGTCC ACAAGTCTGC CTTCAATGAC TGGTTCAGTG GACACCTCAA 240
 CTTCCAGATT GAGCACCATC TTTTTCACAC GATGCCTCGA CACAATTACC ACAAAGTGGC 300
 55 TCCCCTGGTG CAGTCCTTGT GTGCCAAGCA TGGCATAGAG TACCAGTCCA AGCCCCTGCT 360
 GTCAGCCTTC GCCGACATCA TCCACTCACT AAAGGAGTCA GGGCAGCTCT GGCTAGATGC 420
 CTATCTTCAC CAATAACAAC AGCCACCCTG CCCAGTCTGG AAGAAGAGGA GGAAGACTCT 480
 60 GGAGCCAAGG CAGAGGGGAG CTTGAGGGAC AATGCCACTA TAGTTTAATA CTCAGAGGGG 540
 GTTGGGTTTG GGGACATAAA GCCTCTGACT CAACTCCTC CCTTTTATCT TCTAGCCACA 600

GTTCTAAGAC CCAAAGTGGG GGGTGGACAC AGAAGTCCCT AGGAGGGAAG GAGCT 655

5 (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 304 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3506132)

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCTTTTACT TTGGCAATGG CTGGATTCCT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60
 TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120
 CCAAAGTGGG ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCTCTGCC 180
 AACTGGTGGG ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240
 CCCGATGTGA ACATGCTGCA CGTGTTTGTG CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300
 AAGA 304

30 (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 918 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3854933)

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAGGGACCTA CCCC GCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGCAG 60
 GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120
 CCAGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180
 GCCTTCCACA TCAACAAGGG CTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240
 CTGTCTCCAG AGCAGCCCAG CTTTGAGCCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300
 CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360
 CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CTTTGGGTC 420
 TTTGGGACGT CCTTTTGCC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGGCC 480
 CAGGCTGGCT GGCTGCAGCA TGACTTTGGG CACCTGTCGG TCTTCAGCAC CTCAAAGTGG 540
 AACCATCTGC TACATCATTT TGTGATTGGC CACCTGAAGG GGGCCCCCGC CAGTTGGTGG 600
 AACCACATGC ACTTCCAGCA CCATGCCAAG CCCAACTGCT TCCGCAAAGA CCCAGACATC 660

AACATGCATC CCTTCTTCTT TGCCTTGGGG AAGATCCTCT CTGTGGAGCT TGGGAAACAG 720
 5 AAGAAAAAAT ATATGCCGTA CAACCACCAG CACARATACT TCTTCCTAAT TGGGCCCCCA 780
 GCCTTGCTGC CTCTCTACTT CCAGTGGTAT ATTTTCTATT TTGTTATCCA GCGAAAGAAG 840
 TGGGTGGACT TGGCCTGGAT CAGCAAACAG GAATACGATG AAGCCGGGCT TCCATTGTCC 900
 10 ACCGCAAATG CTTCTAAA 918

(2) INFORMATION FOR SEQ ID NO:35:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1686 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 20 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2511785)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

25 GCCACTTAAA GGGTGCCTCT GCCAACTGGT GGAATCATCG CCACTTCCAG CACCACGCCA 60
 AGCCTAACAT CTTCCACAAG GATCCCGATG TGAACATGCT GCACGTGTTT GTTCTGGGCG 120
 30 AATGGCAGCC CATCGAGTAC GGCAAGAAGA AGCTGAAATA CCTGCCCTAC AATCACCAGC 180
 ACGAATACTT CTTCTGATT GGGCCGCCGC TGCTCATCCC CATGTATTTC CAGTACCAGA 240
 35 TCATCATGAC CATGATCGTC CATAAGAACT GGGTGGACCT GGCCTGGGCC GTCAGCTACT 300
 ACATCCGGTT CTTTCATCACC TACATCCCTT TCTACGGCAT CCTGGGAGCC CTCCTTTTCC 360
 TCAACTTCAT CAGGTTCTCTG GAGAGCCACT GGTTTGTGTG GGTCACACAG ATGAATCACA 420
 40 TCGTCATGGA GATTGACCAG GAGGCCTACC GTGACTGGTT CAGTAGCCAG CTGACAGCCA 480
 CCTGCAACGT GGAGCAGTCC TTCTTCAACG ACTGGTTCAG TGGACACCTT AACTTCCAGA 540
 45 TTGAGCACCA CCTCTTCCCC ACCATGCCCC GGCACAACTT ACACAAGATC GCCCCGCTGG 600
 TGAAGTCTCT ATGTGCCAAG CATGGCATTG AATACCAGGA GAAGCCGCTA CTGAGGGCCC 660
 TGCTGGACAT CATCAGGTCC CTGAAGAAGT CTGGGAAGCT GTGGCTGGAC GCCTACCTTC 720
 50 ACAAATGAAG CCACAGCCCC CGGGACACCG TGGGGAAGGG GTGCAGGTGG GGTGATGGCC 780
 AGAGGAATGA TGGGCTTTTG TTCTGAGGGG TGTCCGAGAG GCTGGTGTAT GCACTGCTCA 840
 55 CGGACCCCAT GTTGGATCTT TCTCCCTTTC TCCTCTCCTT TTTCTCTTCA CATCTCCCCC 900
 ATAGCACCTT GCCCTCATGG GACCTGCCCT CCCTCAGCCG TCAGCCATCA GCCATGGCCC 960
 TCCCAGTGCC TCCTAGCCCC TTCTTCCAAG GAGCAGAGAG GTGGCCACCG GGGGTGGCTC 1020
 60 TGTCTACCT CCACTCTCTG CCCCTAAAGA TGGGAGGAGA CCAGCGGTCC ATGGGTCTGG 1080
 CCTGTGAGTC TCCCCTTGCA GCCTGGTCAC TAGGCATCAC CCCCCTTTG GTTCTTCAGA 1140

5 TGCTCTTGGG GTTCATAGGG GCAGGTCTTA GTCGGGCAGG GCCCCTGACC CTCCCGGCCT 1200
 GGCTTCACTC TCCCTGACGG CTGCCATTGG TCCACCCTTT CATAGAGAGG CCTGCTTTGT 1260
 TACAAAGCTC GGGTCTCCCT CCTGCAGCTC GGTTAAGTAC CCGAGGCCTC TCTTAAGATG 1320
 TCCAGGGCCC CAGGCCCCGCG GGCACAGCCA GCCCAAACCT TGGGCCCTGG AAGAGTCCTC 1380
 10 CACCCCATCA CTAGAGTGCT CTGACCCTGG GCTTTCACGG GCCCCATTCC ACCGCCTCCC 1440
 CAACTTGAGC CTGTGACCTT GGGACCAAAG GGGGAGTCCC TCGTCTCTTG TGA CTCAGCA 1500
 15 GAGGCAGTGG CCACGTTT CAG GGAGGGGCGG GCTGGCCTGG AGGCTCAGCC CACCCTCCAG 1560
 CTTTTCTCA GGGTGTCTTG AGGTCCAAGA TTCTGGAGCA ATCTGACCCT TCTCAAAGG 1620
 CTCTGTTATC AGCTGGGCAG TGCCAGCCAA TCCCTGGCCA TTTGGCCCCA GGGGACGTGG 1680
 20 GCCCTG 1686

(2) INFORMATION FOR SEQ ID NO:36:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1843 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 30 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid (Contig 2535)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

35 GTCTTTTACT TTGGCAATGG CTGGATTCCT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60
 TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120
 40 CCCAAGTGGA ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCTCTGCC 180
 AACTGGTGGA ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240
 45 CCCGATGTGA ACATGCTGCA CGTGTTTGTT CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300
 AAGAAGAAGC TGAATACCT GCCCTACAAT CACCAGCACG AATACTTCTT CCTGATTGGG 360
 CCGCCGCTGC TCATCCCAT GTATTTCCAG TACCAGATCA TCATGACCAT GATCGTCCAT 420
 50 AAGAACTGGG TGGACCTGGC CTGGGCGGTC AGCTACTACA TCCGGTTCTT CATCACCTAC 480
 ATCCCTTTCT ACGGCATCCT GGGAGCCCTC CTTTTCTCA ACTTCATCAG GTTCCTGGAG 540
 55 AGCCACTGGT TTGTGTGGGT CACACAGATG AATCACATCG TCATGGAGAT TGACCAGGAG 600
 GCCTACCGTG ACTGGTTCAG TAGCCAGCTG ACAGCCACCT GCAACGTGGA GCAGTCCTTC 660
 TTCAACGACT GGTTCAGTGG ACACCTTAAC TTCCAGATTG AGCACCACCT CTTCCCCACC 720
 60 ATGCCCCGGC ACAACTTACA CAAGATCGCC CCGCTGGTGA AGTCTCTATG TGCCAAGCAT 780
 GGCATTGAAT ACCAGGAGAA GCCGCTACTG AGGGCCCTGC TGGACATCAT CAGGTCCCTG 840

5 AAGAAGTCTG GGAAGCTGTG GCTGGACGCC TACCTTCACA AATGAAGCCA CAGCCCCCGG 900
 GACACCGTGG GGAAGGGGTG CAGGTGGGGT GATGGCCAGA GGAATGATGG GCTTTTGTTC 960
 TGAGGGGTGT CCGAGAGGCT GGTGTATGCA CTGCTCACGG ACCCCATGTT GGATCTTTCT 1020
 CCCTTTCTCC TCTCCTTTTT CTCTTCACAT CTCCCCCATA GCACCCTGCC CTCATGGGAC 1080
 10 CTGCCCTCCC TCAGCCGTCA GCCATCAGCC ATGGCCCTCC CAGTGCCTCC TAGCCCCTTC 1140
 TTCCAAGGAG CAGAGAGGTG GCCACCGGGG GTGGCTCTGT CCTACCTCCA CTCTCTGCCC 1200
 15 CTAAAGATGG GAGGAGACCA GCGGTCCATG GGTCTGGCCT GTGAGTCTCC CTTGTCAGCC 1260
 TGGTCACTAG GCATCACCCC CGCTTTGGTT CTTTCAGATGC TCTTGGGGTT CATAGGGGCA 1320
 GGTCTAGTGC GGGCAGGGCC CCTGACCCTC CCGGCCTGGC TTCACTCTCC CTGACGGCTG 1380
 20 CCATTGGTCC ACCCTTTCAT AGAGAGGCCT GCTTTGTTAC AAAGCTCGGG TCTCCCTCCT 1440
 GCAGCTCGGT TAAGTACCCG AGGCCTCTCT TAAGATGTCC AGGGCCCCAG GCCCGCGGGC 1500
 ACAGCCAGCC CAAACCTTGG GCCCTGGAAG AGTCCTCCAC CCCATCACTA GAGTGCTCTG 1560
 25 ACCCTGGGCT TTCACGGGCC CCATTCCACC GCCTCCCCAA CTTGAGCCTG TGACCTTGGG 1620
 ACCAAAGGGG GAGTCCCTCG TCTCTTGTGA CTCAGCAGAG GCAGTGGCCA CGTTCAGGGA 1680
 30 GGGGCCGGCT GGCCTGGAGG CTCAGCCCAC CCTCCAGCTT TTCCTCAGGG TGTCCTGAGG 1740
 TCCAAGATTC TGGAGCAATC TGACCCTTCT CCAAAGGCTC TGTATCAGC TGGGCAGTGC 1800
 35 CAGCCAATCC CTGGCCATTT GGCCCCAGGG GACGTGGGCC CTG 1843

(2) INFORMATION FOR SEQ ID NO:37:

40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2257 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 45 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 253538a)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
 50 CAGGGACCTA CCCGCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGCGAG 60
 GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120
 CCAGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180
 55 GCCTTCCACA TCAACAAGGG CCTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240
 CTGTCTCCAG AGCAGCCCAG CTTTGTAGCCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300
 CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360
 60 CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CTTTGGGTC 420

| | | |
|----|---|------|
| | TTTGGGACGT CCTTTTGGCC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGCAG | 480 |
| | GCCCAAGCTG GATGGCTGCA ACATGATTAT GGCCACCTGT CTGTCTACAG AAAACCCAAG | 540 |
| 5 | TGGAACCACC TTGTCCACAA ATTCGTCATT GGCCACTTAA AGGGTGCCTC TGCCAACTGG | 600 |
| | TGGAATCATC GCCACTTCCA GCACCAGCC AAGCCTAACA TCTTCCACAA GGATCCCGAT | 660 |
| 10 | GTGAACATGC TGCACGTGTT TGTTCTGGGC GAATGGCAGC CCATCGAGTA CGGCAAGAAG | 720 |
| | AAGCTGAAAT ACCTGCCCTA CAATCACCAG CACGAATACT TCTTCCTGAT TGGGCCGCCG | 780 |
| | CTGCTCATCC CCATGTATTT CCAGTACCAG ATCATCATGA CCATGATCGT CCATAAGAAC | 840 |
| 15 | TGGGTGGACC TGGCCTGGGC CGTCAGCTAC TACATCCGGT TCTTCATCAC CTACATCCCT | 900 |
| | TTCTACGGCA TCCTGGGAGC CCTCCTTTTC CTCAACTTCA TCAGGTTTCT GGAGAGCCAC | 960 |
| 20 | TGGTTTGTGT GGGTCACACA GATGAATCAC ATCGTCATGG AGATTGACCA GGAGGCCTAC | 1020 |
| | CGTGAATGGT TCAGTAGCCA GCTGACAGCC ACCTGCAACG TGGAGCAGTC CTTCTTCAAC | 1080 |
| | GACTGGTTCA GTGGACACCT TAACTTCCAG ATTGAGCACC ACCTCTTCCC CACCATGCCC | 1140 |
| 25 | CGGCACAAC TACACAAGAT CGCCCCGCTG GTGAAGTCTC TATGTGCCAA GCATGGCATT | 1200 |
| | GAATACCAGG AGAAGCCGCT ACTGAGGGCC CTGCTGGACA TCATCAGGTC CCTGAAGAAG | 1260 |
| 30 | TCTGGGAAGC TGTGGCTGGA CGCCTACCTT CACAAATGAA GCCACAGCCC CCGGGACACC | 1320 |
| | GTGGGGAAGG GGTGCAGGTG GGGTGATGGC CAGAGGAATG ATGGGCTTTT GTTCTGAGGG | 1380 |
| | GTGTCCGAGA GGCTGGTGTA TGCACGTCTC ACGGACCCCA TGTTGGATCT TTCTCCCTTT | 1440 |
| 35 | CTCCTCTCCT TTTTCTCTTC ACATCTCCCC CATAGCAGCC TGCCCTCATG GGACCTGCCC | 1500 |
| | TCCCTCAGCC GTCAGCCATC AGCCATGGCC CTCCCAGTGC CTCCTAGCCC CTTCTTCCAA | 1560 |
| 40 | GGAGCAGAGA GGTGGCCACC GGGGGTGGCT CTGTCCTACC TCCACTCTCT GCCCCTAAAG | 1620 |
| | ATGGGAGGAG ACCAGCGGTC CATGGGTCTG GCCTGTGAGT CTCCCCTTGC AGCCTGGTCA | 1680 |
| | CTAGGCATCA CCCCCGCTTT GGTTCCTCAG ATGCTCTTGG GGTTCATAGG GGCAGGTCCT | 1740 |
| 45 | AGTCGGGCAG GGCCCTGAC CCTCCCGGCC TGGCTTCACT CTCCCTGACG GCTGCCATTG | 1800 |
| | GTCCACCCTT TCATAGAGAG GCCTGCTTTG TTACAAAGCT CGGGTCTCCC TCCTGCAGCT | 1860 |
| 50 | CGGTTAAGTA CCCGAGGCCT CTCTTAAGAT GTCCAGGGCC CCAGGCCCGC GGGCACAGCC | 1920 |
| | AGCCCAAACC TTGGGCCCTG GAAGAGTCCT CCACCCATC ACTAGAGTGC TCTGACCCTG | 1980 |
| | GGCTTTACAG GGCCCCATTC CACCGCCTCC CCAACTTGAG CCTGTGACCT TGGGACCAAA | 2040 |
| 55 | GGGGGAGTCC CTCGTCTCTT GTGACTCAGC AGAGGCAGTG GCCACGTTCA GGGAGGGGCC | 2100 |
| | GGCTGGCCTG GAGGCTCAGC CCACCCTCCA GCTTTTCCTC AGGGTGTCTT GAGGTCCAAG | 2160 |
| 60 | ATTCTGGAGC AATCTGACCC TTCTCCAAAG GCTCTGTTAT CAGCTGGGCA GTGCCAGCCA | 2220 |
| | ATCCCTGGCC ATTTGGCCCC AGGGGACGTG GGCCCTG | 2257 |

(2) INFORMATION FOR SEQ ID NO:38:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 411 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: amino acid (Translation of Contig 2692004)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

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15 His Ala Asp Arg Arg Arg Glu Ile Leu Ala Lys Tyr Pro Glu Ile
   1           5           10           15
Lys Ser Leu Met Lys Pro Asp Pro Asn Leu Ile Trp Ile Ile Ile
   20           25           30
Met Met Val Leu Thr Gln Leu Gly Ala Phe Tyr Ile Val Lys Asp
   35           40           45
Leu Asp Trp Lys Trp Val Ile Phe Gly Ala Tyr Ala Phe Gly Ser
   50           55           60
Cys Ile Asn His Ser Met Thr Leu Ala Ile His Glu Ile Ala His
   65           70           75
25 Asn Ala Ala Phe Gly Asn Cys Lys Ala Met Trp Asn Arg Trp Phe
   80           85           90
Gly Met Phe Ala Asn Leu Pro Ile Gly Ile Pro Tyr Ser Ile Ser
   95          100          105
30 Phe Lys Arg Tyr His Met Asp His His Arg Tyr Leu Gly Ala Asp
   110          115          120
Gly Val Asp Val Asp Ile Pro Thr Asp Phe Glu Gly Trp Phe Phe
   125          130          135
Cys Thr Ala Phe Arg Lys Phe Ile Trp Val Ile Leu Gln Pro Leu
   140          145          150
35 Phe Tyr Ala Phe Arg Pro Leu Phe Ile Asn Pro Lys Pro Ile Thr
   155          160          165
Tyr Leu Glu Val Ile Asn Thr Val Ala Gln Val Thr Phe Asp Ile
   170          175          180
Leu Ile Tyr Tyr Phe Leu Gly Ile Lys Ser Leu Val Tyr Met Leu
   185          190          195
40 Ala Ala Ser Leu Leu Gly Leu Gly Leu His Pro Ile Ser Gly His
   200          205          210
Phe Ile Ala Glu His Tyr Met Phe Leu Lys Gly His Glu Thr Tyr
   215          220          225
45 Ser Tyr Tyr Gly Pro Leu Asn Leu Leu Thr Phe Asn Val Gly Tyr
   230          235          240
His Asn Glu His His Asp Phe Pro Asn Ile Pro Gly Lys Ser Leu
   245          250          255
50 Pro Leu Val Arg Lys Ile Ala Ala Glu Tyr Tyr Asp Asn Leu Pro
   260          265          270
His Tyr Asn Ser Trp Ile Lys Val Leu Tyr Asp Phe Val Met Asp
   275          280          285
Asp Thr Ile Ser Pro Tyr Ser Arg Met Lys Arg His Gln Lys Gly
   290          295          300
55 Glu Met Val Leu Glu *** Ile Ser Leu Val Pro Lys Gly Phe Phe
   305          310          315
Ser Lys Thr Leu Asp Asp Lys Met Glu Phe Leu His Tyr *** Thr
   320          325          330
60 *** Asp Gln *** Cys Ser Glu Ala Pro Leu Ala Gln Phe Gln Ser
   335          340          345
Lys Ser Ser Val Ile Pro Arg Ser Glu Ser Gly Phe *** Thr Val
   350          355          360

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Ser Leu Thr Leu Tyr Cys Ser Val Ser Leu Thr Gly Asn Leu ***
 365 370 375
 Leu Val Tyr Tyr Arg His *** Gly Cys Phe Thr His Val Cys His
 380 385 390
 5 Phe Ile Ser Ile Ser Phe Lys Lys Leu Leu Lys Ser Tyr Phe Ala
 400 405 410
 Arg

10 (2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 218 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2153526)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Tyr Leu Leu Arg Pro Leu Leu Pro His Leu Cys Ala Thr Ile Gly
 1 5 10 15
 25 Ala Glu Ser Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu
 20 25 30
 Ser Asn Trp Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met
 35 40 45
 His Ile Asp His Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu
 50 55 60
 30 Gln Ala Thr Cys Asn Val His Lys Ser Ala Phe Asn Asp Trp Phe
 65 70 75
 Ser Gly His Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr
 80 85 90
 35 Met Pro Arg His Asn Tyr His Lys Val Ala Pro Leu Val Gln Ser
 95 100 105
 Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser Lys Pro Leu Leu
 110 115 120
 Ser Ala Phe Ala Asp Ile Ile His Ser Leu Lys Glu Ser Gly Gln
 125 130 135
 40 Leu Trp Leu Asp Ala Tyr Leu His Gln *** Gln Gln Pro Pro Cys
 140 145 150
 Pro Val Trp Lys Lys Arg Arg Lys Thr Leu Glu Pro Arg Gln Arg
 155 160 165
 45 Gly Ala *** Gly Thr Met Pro Leu *** Phe Asn Thr Gln Arg Gly
 170 175 180
 Leu Gly Leu Gly Thr *** Ser Leu *** Leu Lys Leu Leu Pro Phe
 185 190 195
 Ile Phe *** Pro Gln Phe *** Asp Pro Lys Trp Gly Val Asp Thr
 200 205 210
 50 Glu Val Pro Arg Arg Glu Gly Ala
 215

55 (2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71 amino acids
 (B) TYPE: amino acid
 60 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 3506132)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

5

Val Phe Tyr Phe Gly Asn Gly Trp Ile Pro Thr Leu Ile Thr Ala
1 5 10 15
Phe Val Leu Ala Thr Ser Gln Ala Gln Ala Gly Trp Leu Gln His
10 20 25 30
Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys Trp Asn His
35 40 45
Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly Ala Ser Ala
50 55 60
15 Asn Trp Trp Asn His Arg His Phe Gln His His Ala Lys Pro Asn
65 70 75
Leu Gly Glu Trp Gln Pro Ile Glu Tyr Gly Lys Xxx
80 85

20

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 306 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: amino acid (Translation of Contig 3854933)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

35 Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln
1 5 10 15
Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val
20 25 30
40 Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg
35 40 45
Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val
50 55 60
Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser
65 70 75
45 Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro
80 85 90
Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala
95 100 105
Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe
110 115 120
50 Leu Leu Tyr Leu Leu His Ile Leu Leu Asp Gly Ala Ala Trp
125 130 135
Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu
140 145 150
55 Cys Ala Val Leu Leu Ser Ala Val Gln Ala Gln Ala Gly Trp Leu
155 160 165
Gln His Asp Phe Gly His Leu Ser Val Phe Ser Thr Ser Lys Trp
170 175 180
Asn His Leu Leu His His Phe Val Ile Gly His Leu Lys Gly Ala
185 190 195
60 Pro Ala Ser Trp Trp Asn His Met His Phe Gln His His Ala Lys
200 205 210

5 Pro Asn Cys Phe Arg Lys Asp Pro Asp Ile Asn Met His Pro Phe
 215 220 225
 Phe Phe Ala Leu Gly Lys Ile Leu Ser Val Glu Leu Gly Lys Gln
 230 235 240
 10 Lys Lys Lys Tyr Met Pro Tyr Asn His Gln His Xxx Tyr Phe
 245 250 255
 Leu Ile Gly Pro Pro Ala Leu Leu Pro Leu Tyr Phe Gln Trp Tyr
 260 265 270
 15 Ile Phe Tyr Phe Val Ile Gln Arg Lys Lys Trp Val Asp Leu Ala
 275 280 285
 Trp Ile Ser Lys Gln Glu Tyr Asp Glu Ala Gly Leu Pro Leu Ser
 290 295 300
 Thr Ala Asn Ala Ser Lys
 305

(2) INFORMATION FOR SEQ ID NO:42:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 566 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: amino acid (Translation of Contig 2511785)
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

30 His Leu Lys Gly Ala Ser Ala Asn Trp Trp Asn His Arg His Phe
 1 5 10 15
 Gln His His Ala Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val
 20 25 30
 35 Asn Met Leu His Val Phe Val Leu Gly Glu Trp Gln Pro Ile Glu
 35 40 45
 Tyr Gly Lys Lys Lys Leu Lys Tyr Leu Pro Tyr Asn His Gln His
 50 55 60
 Glu Tyr Phe Phe Leu Ile Gly Pro Pro Leu Leu Ile Pro Met Tyr
 65 70 75
 40 Phe Gln Tyr Gln Ile Ile Met Thr Met Ile Val His Lys Asn Trp
 80 85 90
 Val Asp Leu Ala Trp Ala Val Ser Tyr Tyr Ile Arg Phe Phe Ile
 95 100 105
 45 Thr Tyr Ile Pro Phe Tyr Gly Ile Leu Gly Ala Leu Leu Phe Leu
 110 115 120
 Asn Phe Ile Arg Phe Leu Glu Ser His Trp Phe Val Trp Val Thr
 125 130 135
 Gln Met Asn His Ile Val Met Glu Ile Asp Gln Glu Ala Tyr Arg
 140 145 150
 50 Asp Trp Phe Ser Ser Gln Leu Thr Ala Thr Cys Asn Val Glu Gln
 155 160 165
 Ser Phe Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile
 170 175 180
 55 Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu His Lys
 185 190 195
 Ile Ala Pro Leu Val Lys Ser Leu Cys Ala Lys His Gly Ile Glu
 200 205 210
 Tyr Gln Glu Lys Pro Leu Leu Arg Ala Leu Leu Asp Ile Ile Arg
 215 220 225
 60 Ser Leu Lys Lys Ser Gly Lys Leu Trp Leu Asp Ala Tyr Leu His
 230 235 240
 Lys *** Ser His Ser Pro Arg Asp Thr Val Gly Lys Gly Cys Arg

| | | | | | | |
|----|-----------------|---------------------|---------------------|-----|--|-----|
| | | 245 | | 250 | | 255 |
| | Trp Gly Asp Gly | Gln Arg Asn Asp Gly | Leu Leu Phe *** Gly | Val | | |
| | | 260 | | 265 | | 270 |
| 5 | Ser Glu Arg Leu | Val Tyr Ala Leu Leu | Thr Asp Pro Met Leu | Asp | | |
| | | 275 | | 280 | | 285 |
| | Leu Ser Pro Phe | Leu Leu Ser Phe Phe | Ser Ser His Leu Pro | His | | |
| | | 290 | | 295 | | 300 |
| | Ser Thr Leu Pro | Ser Trp Asp Leu Pro | Ser Leu Ser Arg Gln | Pro | | |
| | | 305 | | 310 | | 315 |
| 10 | Ser Ala Met Ala | Leu Pro Val Pro Pro | Ser Pro Phe Phe Gln | Gly | | |
| | | 320 | | 325 | | 330 |
| | Ala Glu Arg Trp | Pro Pro Gly Val Ala | Leu Ser Tyr Leu His | Ser | | |
| | | 335 | | 340 | | 345 |
| 15 | Leu Pro Leu Lys | Met Gly Gly Asp Gln | Arg Ser Met Gly Leu | Ala | | |
| | | 350 | | 355 | | 360 |
| | Cys Glu Ser Pro | Leu Ala Ala Trp Ser | Leu Gly Ile Thr Pro | Ala | | |
| | | 365 | | 370 | | 375 |
| | Leu Val Leu Gln | Met Leu Leu Gly Phe | Ile Gly Ala Gly Pro | Ser | | |
| | | 380 | | 385 | | 390 |
| 20 | Arg Ala Gly Pro | Leu Thr Leu Pro Ala | Trp Leu His Ser Pro | *** | | |
| | | 400 | | 405 | | 410 |
| | Arg Leu Pro Leu | Val His Pro Phe Ile | Glu Arg Pro Ala Leu | Leu | | |
| | | 415 | | 420 | | 425 |
| 25 | Gln Ser Ser Gly | Leu Pro Pro Ala Ala | Arg Leu Ser Thr Arg | Gly | | |
| | | 430 | | 435 | | 440 |
| | Leu Ser *** Asp | Val Gln Gly Pro Arg | Pro Ala Gly Thr Ala | Ser | | |
| | | 445 | | 450 | | 455 |
| | Pro Asn Leu Gly | Pro Trp Lys Ser Pro | Pro Pro His His *** | Ser | | |
| | | 460 | | 465 | | 470 |
| 30 | Ala Leu Thr Leu | Gly Phe His Gly Pro | His Ser Thr Ala Ser | Pro | | |
| | | 475 | | 480 | | 485 |
| | Thr *** Ala Cys | Asp Leu Gly Thr Lys | Gly Gly Val Pro Arg | Leu | | |
| | | 490 | | 495 | | 500 |
| 35 | Leu *** Leu Ser | Arg Gly Ser Gly His | Val Gln Gly Gly Ala | Gly | | |
| | | 505 | | 510 | | 515 |
| | Trp Pro Gly Gly | Ser Ala His Pro Pro | Ala Phe Pro Gln Gly | Val | | |
| | | 520 | | 525 | | 530 |
| | Leu Arg Ser Lys | Ile Leu Glu Gln Ser | Asp Pro Ser Pro Lys | Ala | | |
| | | 535 | | 540 | | 545 |
| 40 | Leu Leu Ser Ala | Gly Gln Cys Gln Pro | Ile Pro Gly His Leu | Ala | | |
| | | 550 | | 555 | | 560 |
| | Pro Gly Asp Val | Gly Pro Xxx | | | | |
| | | 565 | | | | |

45

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 619 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2535)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

60

| | | |
|---|---|----|
| Val Phe Tyr Phe Gly Asn Gly Trp Ile Pro Thr Leu Ile Thr Ala | | |
| 1 | 5 | 10 |
| Phe Val Leu Ala Thr Ser Gln Ala Gln Ala Gly Trp Leu Gln His | | |
| | | 15 |

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | 20 | | | | | 25 | | | | 30 | | |
| | Asp | Tyr | Gly | His | Leu | Ser | Val | Tyr | Arg | Lys | Pro | Lys | Trp | Asn | His |
| | | | | 35 | | | | | | 40 | | | | | 45 |
| 5 | Leu | Val | His | Lys | Phe | Val | Ile | Gly | His | Leu | Lys | Gly | Ala | Ser | Ala |
| | | | | 50 | | | | | | 55 | | | | | 60 |
| | Asn | Trp | Trp | Asn | His | Arg | His | Phe | Gln | His | His | Ala | Lys | Pro | Asn |
| | | | | 65 | | | | | | 70 | | | | | 75 |
| | Ile | Phe | His | Lys | Asp | Pro | Asp | Val | Asn | Met | Leu | His | Val | Phe | Val |
| | | | | 80 | | | | | | 85 | | | | | 90 |
| 10 | Leu | Gly | Glu | Trp | Gln | Pro | Ile | Glu | Tyr | Gly | Lys | Lys | Lys | Leu | Lys |
| | | | | 95 | | | | | | 100 | | | | | 105 |
| | Tyr | Leu | Pro | Tyr | Asn | His | Gln | His | Glu | Tyr | Phe | Phe | Leu | Ile | Gly |
| | | | | 110 | | | | | | 115 | | | | | 120 |
| 15 | Pro | Pro | Leu | Leu | Ile | Pro | Met | Tyr | Phe | Gln | Tyr | Gln | Ile | Ile | Met |
| | | | | 125 | | | | | | 130 | | | | | 135 |
| | Thr | Met | Ile | Val | His | Lys | Asn | Trp | Val | Asp | Leu | Ala | Trp | Ala | Val |
| | | | | 140 | | | | | | 145 | | | | | 150 |
| | Ser | Tyr | Tyr | Ile | Arg | Phe | Phe | Ile | Thr | Tyr | Ile | Pro | Phe | Tyr | Gly |
| | | | | 155 | | | | | | 160 | | | | | 165 |
| 20 | Ile | Leu | Gly | Ala | Leu | Leu | Phe | Leu | Asn | Phe | Ile | Arg | Phe | Leu | Glu |
| | | | | 170 | | | | | | 175 | | | | | 180 |
| | Ser | His | Trp | Phe | Val | Trp | Val | Thr | Gln | Met | Asn | His | Ile | Val | Met |
| | | | | 185 | | | | | | 190 | | | | | 195 |
| 25 | Glu | Ile | Asp | Gln | Glu | Ala | Tyr | Arg | Asp | Trp | Phe | Ser | Ser | Gln | Leu |
| | | | | 200 | | | | | | 205 | | | | | 210 |
| | Thr | Ala | Thr | Cys | Asn | Val | Glu | Gln | Ser | Phe | Phe | Asn | Asp | Trp | Phe |
| | | | | 215 | | | | | | 220 | | | | | 225 |
| | Ser | Gly | His | Leu | Asn | Phe | Gln | Ile | Glu | His | His | Leu | Phe | Pro | Thr |
| | | | | 230 | | | | | | 235 | | | | | 240 |
| 30 | Met | Pro | Arg | His | Asn | Leu | His | Lys | Ile | Ala | Pro | Leu | Val | Lys | Ser |
| | | | | 245 | | | | | | 250 | | | | | 255 |
| | Leu | Cys | Ala | Lys | His | Gly | Ile | Glu | Tyr | Gln | Glu | Lys | Pro | Leu | Leu |
| | | | | 260 | | | | | | 265 | | | | | 270 |
| 35 | Arg | Ala | Leu | Leu | Asp | Ile | Ile | Arg | Ser | Leu | Lys | Lys | Ser | Gly | Lys |
| | | | | 275 | | | | | | 280 | | | | | 285 |
| | Leu | Trp | Leu | Asp | Ala | Tyr | Leu | His | Lys | *** | Ser | His | Ser | Pro | Arg |
| | | | | 290 | | | | | | 295 | | | | | 300 |
| | Asp | Thr | Val | Gly | Lys | Gly | Cys | Arg | Trp | Gly | Asp | Gly | Gln | Arg | Asn |
| | | | | 305 | | | | | | 310 | | | | | 315 |
| 40 | Asp | Gly | Leu | Leu | Phe | *** | Gly | Val | Ser | Glu | Arg | Leu | Val | Tyr | Ala |
| | | | | 320 | | | | | | 325 | | | | | 330 |
| | Leu | Leu | Thr | Asp | Pro | Met | Leu | Asp | Leu | Ser | Pro | Phe | Leu | Leu | Ser |
| | | | | 335 | | | | | | 340 | | | | | 345 |
| 45 | Phe | Phe | Ser | Ser | His | Leu | Pro | His | Ser | Thr | Leu | Pro | Ser | Trp | Asp |
| | | | | 350 | | | | | | 355 | | | | | 360 |
| | Leu | Pro | Ser | Leu | Ser | Arg | Gln | Pro | Ser | Ala | Met | Ala | Leu | Pro | Val |
| | | | | 365 | | | | | | 370 | | | | | 375 |
| | Pro | Pro | Ser | Pro | Phe | Phe | Gln | Gly | Ala | Glu | Arg | Trp | Pro | Pro | Gly |
| | | | | 380 | | | | | | 385 | | | | | 390 |
| 50 | Val | Ala | Leu | Ser | Tyr | Leu | His | Ser | Leu | Pro | Leu | Lys | Met | Gly | Gly |
| | | | | 400 | | | | | | 405 | | | | | 410 |
| | Asp | Gln | Arg | Ser | Met | Gly | Leu | Ala | Cys | Glu | Ser | Pro | Leu | Ala | Ala |
| | | | | 415 | | | | | | 420 | | | | | 425 |
| 55 | Trp | Ser | Leu | Gly | Ile | Thr | Pro | Ala | Leu | Val | Leu | Gln | Met | Leu | Leu |
| | | | | 430 | | | | | | 435 | | | | | 440 |
| | Gly | Phe | Ile | Gly | Ala | Gly | Pro | Ser | Arg | Ala | Gly | Pro | Leu | Thr | Leu |
| | | | | 445 | | | | | | 450 | | | | | 455 |
| | Pro | Ala | Trp | Leu | His | Ser | Pro | *** | Arg | Leu | Pro | Leu | Val | His | Pro |
| | | | | 460 | | | | | | 465 | | | | | 470 |
| 60 | Phe | Ile | Glu | Arg | Pro | Ala | Leu | Leu | Gln | Ser | Ser | Gly | Leu | Pro | Pro |
| | | | | 475 | | | | | | 480 | | | | | 485 |
| | Ala | Ala | Arg | Leu | Ser | Thr | Arg | Gly | Leu | Ser | *** | Asp | Val | Gln | Gly |

490 495 500
 Pro Arg Pro Ala Gly Thr Ala Ser Pro Asn Leu Gly Pro Trp Lys
 505 510 515
 5 Ser Pro Pro Pro His His *** Ser Ala Leu Thr Leu Gly Phe His
 520 525 530
 Gly Pro His Ser Thr Ala Ser Pro Thr *** Ala Cys Asp Leu Gly
 535 540 545
 Thr Lys Gly Gly Val Pro Arg Leu Leu *** Leu Ser Arg Gly Ser
 550 555 560
 10 Gly His Val Gln Gly Gly Ala Gly Trp Pro Gly Gly Ser Ala His
 565 570 575
 Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys Ile Leu Glu
 580 585 590
 15 Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala Gly Gln Cys
 595 600 605
 Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val Gly Pro Xxx
 610 615 620

20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 757 amino acids
 25 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 253538a)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

35 Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln
 1 5 10 15
 Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val
 20 25 30
 Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg
 35 40 45
 40 Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val
 50 55 60
 Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser
 65 70 75
 45 Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro
 80 85 90
 Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala
 95 100 105
 Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe
 110 115 120
 50 Leu Leu Tyr Leu Leu His Ile Leu Leu Leu Asp Gly Ala Ala Trp
 125 130 135
 Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu
 140 145 150
 55 Cys Ala Val Leu Leu Ser Ala Val Gln Gln Ala Gln Ala Gly Trp
 155 160 165
 Leu Gln His Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys
 170 175 180
 Trp Asn His Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly
 185 190 195
 60 Ala Ser Ala Asn Trp Trp Asn His Arg His Phe Gln His His Ala
 200 205 210
 Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val Asn Met Leu His

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | | | 215 | | | | | 220 | | | | | 225 |
| | Val | Phe | Val | Leu | Gly | Glu | Trp | Gln | Pro | Ile | Glu | Tyr | Gly | Lys | Lys |
| | | | | | 230 | | | | | 235 | | | | | 240 |
| 5 | Lys | Leu | Lys | Tyr | Leu | Pro | Tyr | Asn | His | Gln | His | Glu | Tyr | Phe | Phe |
| | | | | | 245 | | | | | 250 | | | | | 255 |
| | Leu | Ile | Gly | Pro | Pro | Leu | Leu | Ile | Pro | Met | Tyr | Phe | Gln | Tyr | Gln |
| | | | | | 260 | | | | | 265 | | | | | 270 |
| | Ile | Ile | Met | Thr | Met | Ile | Val | His | Lys | Asn | Trp | Val | Asp | Leu | Ala |
| | | | | | 275 | | | | | 280 | | | | | 285 |
| 10 | Trp | Ala | Val | Ser | Tyr | Tyr | Ile | Arg | Phe | Phe | Ile | Thr | Tyr | Ile | Pro |
| | | | | | 290 | | | | | 295 | | | | | 300 |
| | Phe | Tyr | Gly | Ile | Leu | Gly | Ala | Leu | Leu | Phe | Leu | Asn | Phe | Ile | Arg |
| | | | | | 305 | | | | | 310 | | | | | 315 |
| 15 | Phe | Leu | Glu | Ser | His | Trp | Phe | Val | Trp | Val | Thr | Gln | Met | Asn | His |
| | | | | | 320 | | | | | 325 | | | | | 330 |
| | Ile | Val | Met | Glu | Ile | Asp | Gln | Glu | Ala | Tyr | Arg | Asp | Trp | Phe | Ser |
| | | | | | 335 | | | | | 340 | | | | | 345 |
| | Ser | Gln | Leu | Thr | Ala | Thr | Cys | Asn | Val | Glu | Gln | Ser | Phe | Phe | Asn |
| | | | | | 350 | | | | | 355 | | | | | 360 |
| 20 | Asp | Trp | Phe | Ser | Gly | His | Leu | Asn | Phe | Gln | Ile | Glu | His | His | Leu |
| | | | | | 365 | | | | | 370 | | | | | 375 |
| | Phe | Pro | Thr | Met | Pro | Arg | His | Asn | Leu | His | Lys | Ile | Ala | Pro | Leu |
| | | | | | 380 | | | | | 385 | | | | | 390 |
| 25 | Val | Lys | Ser | Leu | Cys | Ala | Lys | His | Gly | Ile | Glu | Tyr | Gln | Glu | Lys |
| | | | | | 400 | | | | | 405 | | | | | 410 |
| | Pro | Leu | Leu | Arg | Ala | Leu | Leu | Asp | Ile | Ile | Arg | Ser | Leu | Lys | Lys |
| | | | | | 415 | | | | | 420 | | | | | 425 |
| | Ser | Gly | Lys | Leu | Trp | Leu | Asp | Ala | Tyr | Leu | His | Lys | *** | Ser | His |
| | | | | | 430 | | | | | 435 | | | | | 440 |
| 30 | Ser | Pro | Arg | Asp | Thr | Val | Gly | Lys | Gly | Cys | Arg | Trp | Gly | Asp | Gly |
| | | | | | 445 | | | | | 450 | | | | | 455 |
| | Gln | Arg | Asn | Asp | Gly | Leu | Leu | Phe | *** | Gly | Val | Ser | Glu | Arg | Leu |
| | | | | | 460 | | | | | 465 | | | | | 470 |
| 35 | Val | Tyr | Ala | Leu | Leu | Thr | Asp | Pro | Met | Leu | Asp | Leu | Ser | Pro | Phe |
| | | | | | 475 | | | | | 480 | | | | | 485 |
| | Leu | Leu | Ser | Phe | Phe | Ser | Ser | His | Leu | Pro | His | Ser | Thr | Leu | Pro |
| | | | | | 490 | | | | | 495 | | | | | 500 |
| | Ser | Trp | Asp | Leu | Pro | Ser | Leu | Ser | Arg | Gln | Pro | Ser | Ala | Met | Ala |
| | | | | | 505 | | | | | 510 | | | | | 515 |
| 40 | Leu | Pro | Val | Pro | Pro | Ser | Pro | Phe | Phe | Gln | Gly | Ala | Glu | Arg | Trp |
| | | | | | 520 | | | | | 525 | | | | | 530 |
| | Pro | Pro | Gly | Val | Ala | Leu | Ser | Tyr | Leu | His | Ser | Leu | Pro | Leu | Lys |
| | | | | | 535 | | | | | 540 | | | | | 545 |
| 45 | Met | Gly | Gly | Asp | Gln | Arg | Ser | Met | Gly | Leu | Ala | Cys | Glu | Ser | Pro |
| | | | | | 550 | | | | | 555 | | | | | 560 |
| | Leu | Ala | Ala | Trp | Ser | Leu | Gly | Ile | Thr | Pro | Ala | Leu | Val | Leu | Gln |
| | | | | | 565 | | | | | 570 | | | | | 575 |
| | Met | Leu | Leu | Gly | Phe | Ile | Gly | Ala | Gly | Pro | Ser | Arg | Ala | Gly | Pro |
| | | | | | 580 | | | | | 585 | | | | | 590 |
| 50 | Leu | Thr | Leu | Pro | Ala | Trp | Leu | His | Ser | Pro | *** | Arg | Leu | Pro | Leu |
| | | | | | 595 | | | | | 600 | | | | | 605 |
| | Val | His | Pro | Phe | Ile | Glu | Arg | Pro | Ala | Leu | Leu | Gln | Ser | Ser | Gly |
| | | | | | 610 | | | | | 615 | | | | | 620 |
| 55 | Leu | Pro | Pro | Ala | Ala | Arg | Leu | Ser | Thr | Arg | Gly | Leu | Ser | *** | Asp |
| | | | | | 625 | | | | | 630 | | | | | 635 |
| | Val | Gln | Gly | Pro | Arg | Pro | Ala | Gly | Thr | Ala | Ser | Pro | Asn | Leu | Gly |
| | | | | | 640 | | | | | 645 | | | | | 650 |
| | Pro | Trp | Lys | Ser | Pro | Pro | Pro | His | His | *** | Ser | Ala | Leu | Thr | Leu |
| | | | | | 655 | | | | | 660 | | | | | 665 |
| 60 | Gly | Phe | His | Gly | Pro | His | Ser | Thr | Ala | Ser | Pro | Thr | *** | Ala | Cys |
| | | | | | 670 | | | | | 675 | | | | | 680 |
| | Asp | Leu | Gly | Thr | Lys | Gly | Gly | Val | Pro | Arg | Leu | Leu | *** | Leu | Ser |

```

        685                690                695
Arg Gly Ser Gly His Val Gln Gly Gly Ala Gly Trp Pro Gly Gly
        700                705                710
5  Ser Ala His Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys
        715                720                725
Ile Leu Glu Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala
        730                735                740
Gly Gln Cys Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val
        745                750                755
10 Gly Pro Xxx

```

(2) INFORMATION FOR SEQ ID NO:45:

```

15  (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 746 nucleic acids
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: not relevant
      (D) TOPOLOGY: linear

```

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```

25  CGTATGTCAC TCCATTCCAA ACTCGTTCAT GGTATCATAA ATATCAACAC ATTTACGCTC      60
    CACTCCTCTA TGGTATTTAC ACACTCAAAT ATCGTACTCA AGATTGGGAA GCTTTTGTA      120
    AGGATGGTAA AAATGGTGCA ATTCGTGTTA GTGTCGCCAC AAATTTTCGAT AAGGCCGCTT      180
    ACGTCATTGG TAAATTGTCT TTTGTTTTCT TCCGTTTCAT CCTTCCACTC CGTTATCATA      240
    GCTTTACAGA TTTAATTTGT TATTTCTCTA TTGCTGAATT CGTCTTTGGT TGGTATCTCA      300
30  CAATTAATTT CCAAGTTAGT CATGTCGCTG AAGATCTCAA ATTCTTTGCT ACCCTGAAA      360
    GACCAGATGA ACCATCTCAA ATCAATGAAG ATTGGGCAAT CCTTCAACTT AAAACTACTC      420
    AAGATTATGG TCATGGTTCA CTCCTTTGTA CCTTTTTTAG TGGTCTTTTA AATCATCAAG      480
    TTGTTTCATCA TTTATTCCCA TCAATTGCTC AAGATTTCTA CCCACAACCT GTACCAATTG      540
35  TAAAAGAAGT TTGTAAAGAA CATAACATTA CTTACCAT TAAACCAAAC TTCACTGAAG      600
    CTATTATGTC ACACATTAAT TACCTTTACA AAATGGGTAA TGATCCAGAT TATGTTAAAA      660
    AACCATTAGC CTCAAAAGAT GATTAAATGA AATAACTTAA AAACCAATTA TTTACTTTTG      720
    ACAAACAGTA ATATTAATAA ATACAA                                     746

```

40 (2) INFORMATION FOR SEQ ID NO:46:

```

      (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 227 amino acids
      (B) TYPE: amino acid
45  (C) STRANDEDNESS: not relevant
      (D) TOPOLOGY: linear

```

(ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```

Tyr Val Thr Pro Phe Gln Thr Arg Ser Trp Tyr His Lys Tyr Gln
1  5 10 15
55 His Ile Tyr Ala Pro Leu Leu Tyr Gly Ile Tyr Thr Leu Lys Tyr
    20 25 30
    Arg Thr Gln Asp Trp Glu Ala Phe Val Lys Asp Gly Lys Asn Gly
    35 40 45
    Ala Ile Arg Val Ser Val Ala Thr Asn Phe Asp Lys Ala Ala Tyr
    50 55 60
60 Val Ile Gly Lys Leu Ser Phe Val Phe Phe Arg Phe Ile Leu Pro
    65 70 75
    Leu Arg Tyr His Ser Phe Thr Asp Leu Ile Cys Tyr Phe Leu Ile
    80 85 90
65 Ala Glu Ph Val Phe Gly Trp Tyr Leu Thr Ile Asn Phe Gln Val
    95 100 105

```

Ser His Val Ala Glu Asp Leu Lys Phe Phe Ala Thr Pro Glu Arg
 110 115 120
 Pro Asp Glu Pro Ser Gln Ile Asn Glu Asp Trp Ala Ile Leu Gln
 125 130 135
 5 Leu Lys Thr Thr Gln Asp Tyr Gly His Gly Ser Leu Leu Cys Thr
 140 145 150
 Phe Phe Ser Gly Ser Leu Asn His Gln Val Val His His Leu Phe
 155 160 165
 10 Pro Ser Ile Ala Gln Asp Phe Tyr Pro Gln Leu Val Pro Ile Val
 170 175 180
 Lys Glu Val Cys Lys Glu His Asn Ile Thr Tyr His Ile Lys Pro
 185 190 195
 Asn Phe Thr Glu Ala Ile Met Ser His Ile Asn Tyr Leu Tyr Lys
 200 205 210
 15 Met Gly Asn Asp Pro Asp Tyr Val Lys Lys Pro Leu Ala Ser Lys
 215 220 225
 Asp Asp ***

20 (2) INFORMATION FOR SEQ ID NO 47:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 494 nucleic acids
 25 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTTTGAAGG NTCCAAGTTN ACCACGGANT NGGCAAGTTN ACGGGGCGGA AANCGGTTTT 60
 35 CCCCCCAAGC CTTTGTGCGA CTGGTTCTGT GGTGGCTTCC AGTACCAAGT CGACCACCAC 120
 TTATTCGCCA GCCTGCCCGG ACACAATCTG GCCAAGACAC ACGCACTGGT CGAATCGTTC 180
 TGCAAGGAGT GGGGTGTCCA GTACCACGAA GCCGACCTCG TGGACGGGAC CATGGAAGTC 240
 TTGCACCATT TGGGCAGCGT GGCCGGCGAA TTCGTCGTGG ATTTGTACG CGACGGACCC 300
 GCCATGTAAT CGTCGTTCGT GACGATGCAA GGGTTCACGC ACATCTACAC AACTCACTC 360
 40 ACACAAC TAGTAACTCGT ATAGAATTCG GTGTCGACCT GGACCTTGTT TGA CTGGTTG 420
 GGGATAGGGT AGGTAGGCGG ACGCGTGGGT CGNCCCCGGG AATTCTGTGA CCGGTACCTG 480
 GCCCGCGTNA AAGT 494

45 (2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 87 amino acids
 50 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Phe Trp Lys Xxx Pro Ser Xxx Pro Arg Xxx Xxx Gln Val Xxx Gly
 1 5 10 15
 60 Ala Glu Xxx Gly Phe Pro Pro Lys Pro Phe Val Asp Trp Phe Cys
 20 25 30
 Gly Gly Phe Gln Tyr Gln Val Asp His His Leu Phe Pro Ser Leu
 35 40 45
 Pro Arg His Asn L u Ala Lys Thr His Ala Leu Val Glu Ser Phe
 50 55 60
 65 Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu Val Asp
 65 70 75

Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly Glu
 65 70 75
 Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met
 80 85

5

10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 520 nucleic acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

25 GGATGGAGTT CGTCTGGATC GCTGTGCGCT ACGCGACGTG GTTTAAGCGT CATGGGTGCG 60
 CTTGGGTACA CGCCGGGGCA GTCGTTGGGC ATGTAATTGT GCGCCTTTGG TCTCGGCTGC 120
 ATTTACATTT TTCTGCAGTT CGCCGTAAGT CACACCCATT TGCCCGTGAG CAACCCGGAG 180
 GATCAGCTGC ATTGGCTCGA GTACGCGCGG ACCACACTGT GAACATCAGC ACCAAGTCGT 240
 GGTGTGTCAC ATGGTGGATG TCGAACCTCA ACTTTCAGAT CGAGCACCAC CTTTTCCCCA 300
 CGGCGCCCCA GTTCCGTTTC AAGGAGATCA GCCCGCGCGT CGAGGCCCTC TTCAAGCGCC 360
 30 ACGGTCTCCC TTAACACGAC ATGCCCTACA CGAGCGCCGT CTCCACCACC TTTGCCAACC 420
 TCTACTCCGT CGGCCATTCC GTCGGCGACG CCAAGCGCGA CTAGCCTCTT TTCCTAGACC 480
 TTAATTCCCC ACCCCACCCC ATGTTCTGTC TTCCTCCGCG 520

35

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH: 153 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

50 Met Glu Phe Val Trp Ile Ala Val Arg Tyr Ala Thr Trp Phe Lys
 1 5 10 15
 Arg His Gly Cys Ala Trp Val His Ala Gly Ala Val Val Gly His
 20 25 30
 Val Leu Val Arg Leu Trp Ser Arg Leu His Leu His Phe Ser Ala
 35 40 45
 55 Val Arg Arg Lys Ser His Pro Phe Ala Arg Glu Gln Pro Gly Gly
 50 55 60
 Ser Ala Ala Leu Ala Arg Val Arg Ala Asp His Thr Val Asn Ile
 65 70 75
 Ser Thr Lys Ser Trp Phe Val Thr Trp Trp Met Ser Asn Leu Asn
 80 85 90
 60 Phe Gln Ile Glu His His Leu Phe Pro Thr Ala Pro Gln Phe Arg
 95 100 105
 Ph Lys Glu Ile S r Pro Arg Val Glu Ala Leu Phe Lys Arg His
 110 115 120
 65 Gly Leu Pro Tyr Tyr Asp Met Pro Tyr Thr Ser Ala Val Ser Thr
 125 130 135
 Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly Asp Ala

Lys Arg Asp 140 145 150

5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 429 nucleic acids
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

10

15

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

20

25

```
ACGCGTCCGC CCACGCGTCC GCCGCGAGCA ACTCATCAAG GAAGGCTACT TTGACCCCTC 60
GCTCCCGCAC ATGACGTACC GCGTGGTCGA GATTGTTGTT CTCTTCGTGC TTTCTTTTGG 120
GCTGATGGGT CAGTCTTCAC CCCTCGCGCT CGCTCTCGGC ATTGTCGTCA GCGGCATCTC 180
TCAGGGTCGC TCGGGCTGGG TAATGCATGA GATGGGCCAT GGGTCGTTCA CTGGTGTCAT 240
TTGGCTTGAC GACCGGTTGT GCGAGTTCTT TTACGGCGTT GGTGTGGCA TGAGCGGTCA 300
TTACTGGAAA AACCAGCACA GCAAACACCA CGCAGCGCCA AACCGGCTCG AGCACGATGT 360
AGATCTCAAC ACCTTGCCAT TGGTGGCCTT CAACGAGCGC GTCGTGCGCA AGGTCCGACC 420
```

30

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: not relevant
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

40

45

50

55

60

```
Arg Val Arg Pro Arg Val Arg Arg Glu Gln Leu Ile Lys Glu Gly
1 5 10 15
Tyr Phe Asp Pro Ser Leu Pro His Met Thr Tyr Arg Val Val Glu
20 25 30
Ile Val Val Leu Phe Val Leu Ser Phe Trp Leu Met Gly Gln Ser
35 40 45
Ser Pro Leu Ala Leu Ala Leu Gly Ile Val Val Ser Gly Ile Ser
50 55 60
Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly Ser
65 70 75
Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Leu Cys Glu Phe Phe
65 70 75
Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln
80 85 90
His Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val
95 100 105
Asp Leu Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val
110 115 120
Arg Lys Val Arg Pro
125
```


What is claimed is:

1. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5,
5 wherein said one or more nucleotide sequences is linked to a heterologous nucleotide sequence.

2. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5,
10 wherein said one or more nucleotide sequences is operably associated with an expression control sequence functional in a plant cell.

3. The nucleic acid construct according to claim 2, wherein said nucleotide
15 sequence has an average A + T content of less than about 60%.

4. The nucleic acid construct according to claim 2, wherein said nucleotide
sequence is derived from a fungus.

5. The nucleic acid construct according to claim 4, wherein said fungus is of
20 the genus *Mortierella*.

6. The nucleic acid construct according to claim 5, wherein said fungus is of
the species *alpina*.

7. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino
acid sequence depicted in SEQ ID NO:2, wherein said nucleotide sequence is
25

operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 6 from the carboxyl end of said fatty acid molecule.

5

8. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:4, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence functional in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 12 from the carboxyl end of said fatty acid molecule.

10

9. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 5 from the carboxyl end of said fatty acid molecule.

15

20

10. A nucleic acid construct comprising:

at least one nucleotide sequence which encodes a functionally active desaturase having an amino acid sequence depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a promoter functional in a plant cell.

25

11. The nucleic acid construct according to claim 10, wherein said plant cell is a seed cell.

5 12. The nucleic acid construct according to claim 11, wherein said seed cell is an embryo cell.

13. A recombinant plant cell comprising:

10 At least one copy of a DNA sequence which encodes at least one functionally active *Mortierella alpina* fatty acid desaturase which results in the production of a polyunsaturated fatty acid, wherein said fatty acid desaturase has an amino acid sequence as depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, wherein said cell was transformed with a vector comprising said DNA sequence, and wherein said DNA sequence is operably associated with an expression control
15 sequence.

14. The recombinant plant cell of claim 13, wherein said polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

20

15. The recombinant plant cell of claim 13, wherein said recombinant plant cell is enriched in a fatty acid selected from the group consisting of 18:1, 18:2, 18:3 and 18:4.

25 16. The recombinant plant cell of claim 15, wherein said plant cell is selected from the group consisting of *Brassica*, soybean, safflower, corn, flax, and sunflower.

17. The recombinant plant cell according to claim 16, wherein said expression control sequence is endogenous to said plant cell.

18. One or more plant oils expressed by said recombinant plant cell of claim 16.

5

19. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain a transgene encoding a transgene expression product which desaturates a fatty acid molecule at carbon
10 5 from the carboxyl end of said fatty acid molecule, wherein said transgene is operably associated with an expression control sequence, under conditions whereby said transgene is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

15 20. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain one or more transgenes, derived from a fungus or algae, which encodes a transgene expression product which desaturates a fatty acid molecule at a carbon selected from the group
20 consisting of carbon 5, carbon 6 and carbon 12 from the carboxyl end of said fatty acid molecule, wherein said one or more transgenes is operably associated with an expression control sequence, under conditions whereby said one or more transgenes is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

25

21. The method according to claims 19 or 20, wherein said long chain polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

22. A plant oil or fraction thereof produced according to the method of claims 19 or 20.
- 5 23. A method of treating or preventing malnutrition comprising administering said plant oil of claim 22 to a patient in need of said treatment or prevention in an amount sufficient to effect said treatment or prevention.
24. A pharmaceutical composition comprising said plant oil or fraction of claim 22 and a pharmaceutically acceptable carrier.
- 10 25. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is in the form of a solid or a liquid.
- 15 26. The pharmaceutical composition of claim 25, wherein said pharmaceutical composition is in a capsule or tablet form.
- 20 27. The pharmaceutical composition of claim 24 further comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.
28. A nutritional formula comprising said plant oil or fraction thereof of claim 22.
- 25 29. The nutritional formula of claim 28, wherein said nutritional formula is selected from the group consisting of an infant formula, a dietary supplement, and a dietary substitute.

30. The nutritional formula of claim 29, wherein said infant formula, dietary supplement or dietary supplement is in the form of a liquid or a solid.

31. An infant formula comprising said plant oil or fraction thereof of claim 22.

5

32. The infant formula of claim 31 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

10

33. The infant formula of claim 32 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

15

34. A dietary supplement comprising said plant oil or fraction thereof of claim 22.

20

35. The dietary supplement of claim 34 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

25

36. The dietary supplement of claim 35 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium,

magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

5 37. The dietary supplement of claim 34 or claim 36, wherein said dietary supplement is administered to a human or an animal.

38. A dietary substitute comprising said plant oil or fraction thereof of claim 22.

10 39. The dietary substitute of claim 38 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electro dialysed whey, electro dialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

15 40. The dietary substitute of claim 39 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

20

41. The dietary substitute of claim 38 or claim 40, wherein said dietary substitute is administered to a human or animal.

25 42. A method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to said patient said dietary substitute of claim 38 or said dietary supplement of claim 34 in an amount sufficient to effect said treatment.

43. The method of claim 42, wherein said dietary substitute or said dietary supplement is administered enterally or parenterally.

44. A cosmetic comprising said plant oil or fraction thereof of claim 22.

5

45. The cosmetic of claim 44, wherein said cosmetic is applied topically.

46. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is administered to a human or an animal.

10

47. An animal feed comprising said plant oil or fraction thereof of claim 22.

48. An isolated nucleotide sequence comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:38 - SEQ ID NO:44 wherein said nucleotide sequence is expressed in a plant cell.

15

49. The method of claim 20 wherein said fungus is *Mortierella species*.

50. The method of claim 49 wherein said fungus is *Mortierella alpina*.

20

51. An isolated nucleotide sequence selected from the group consisting of SEQ ID NO:49 - SEQ ID NO:50 wherein said sequence is expressed in a plant cell.

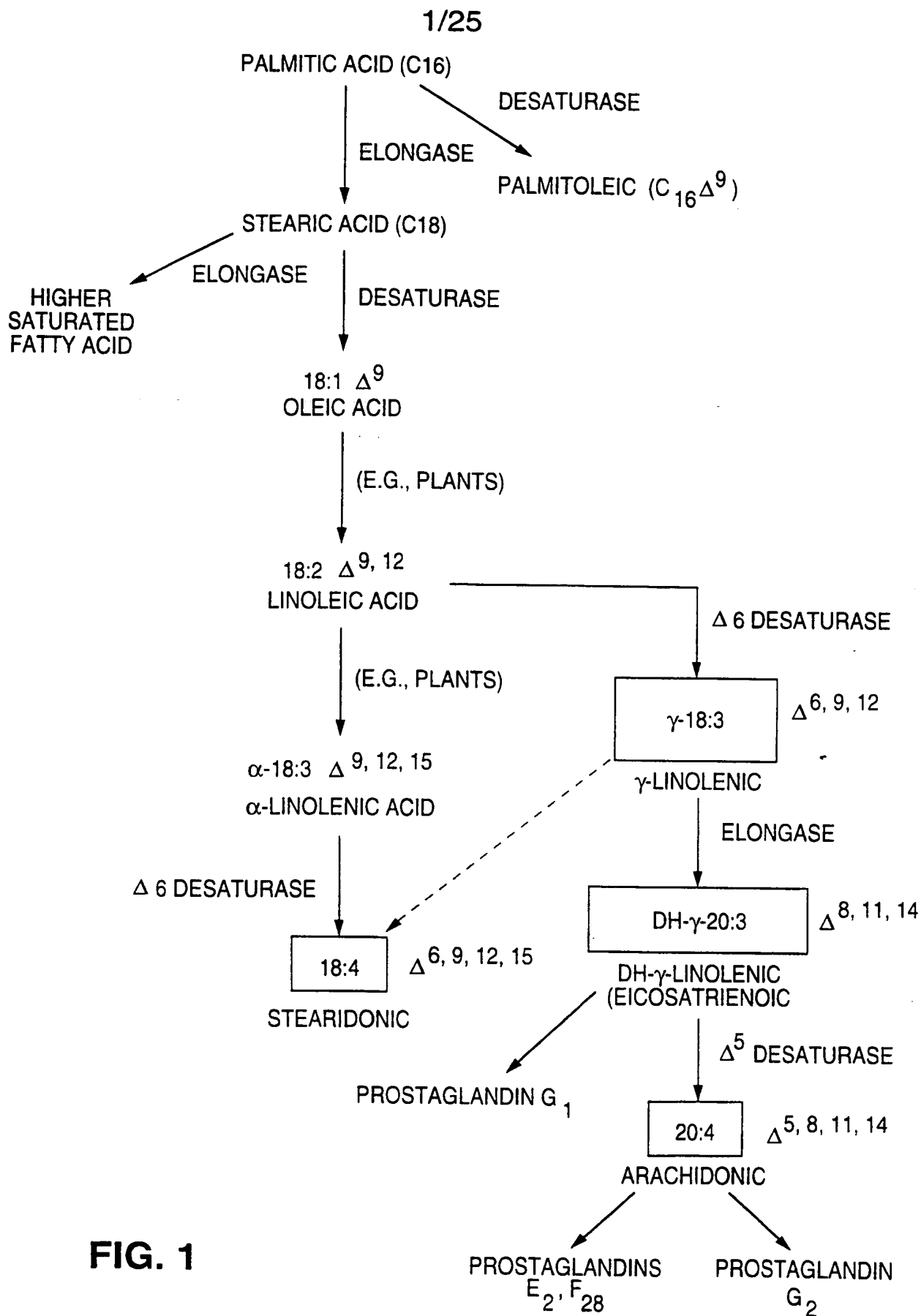


FIG. 1

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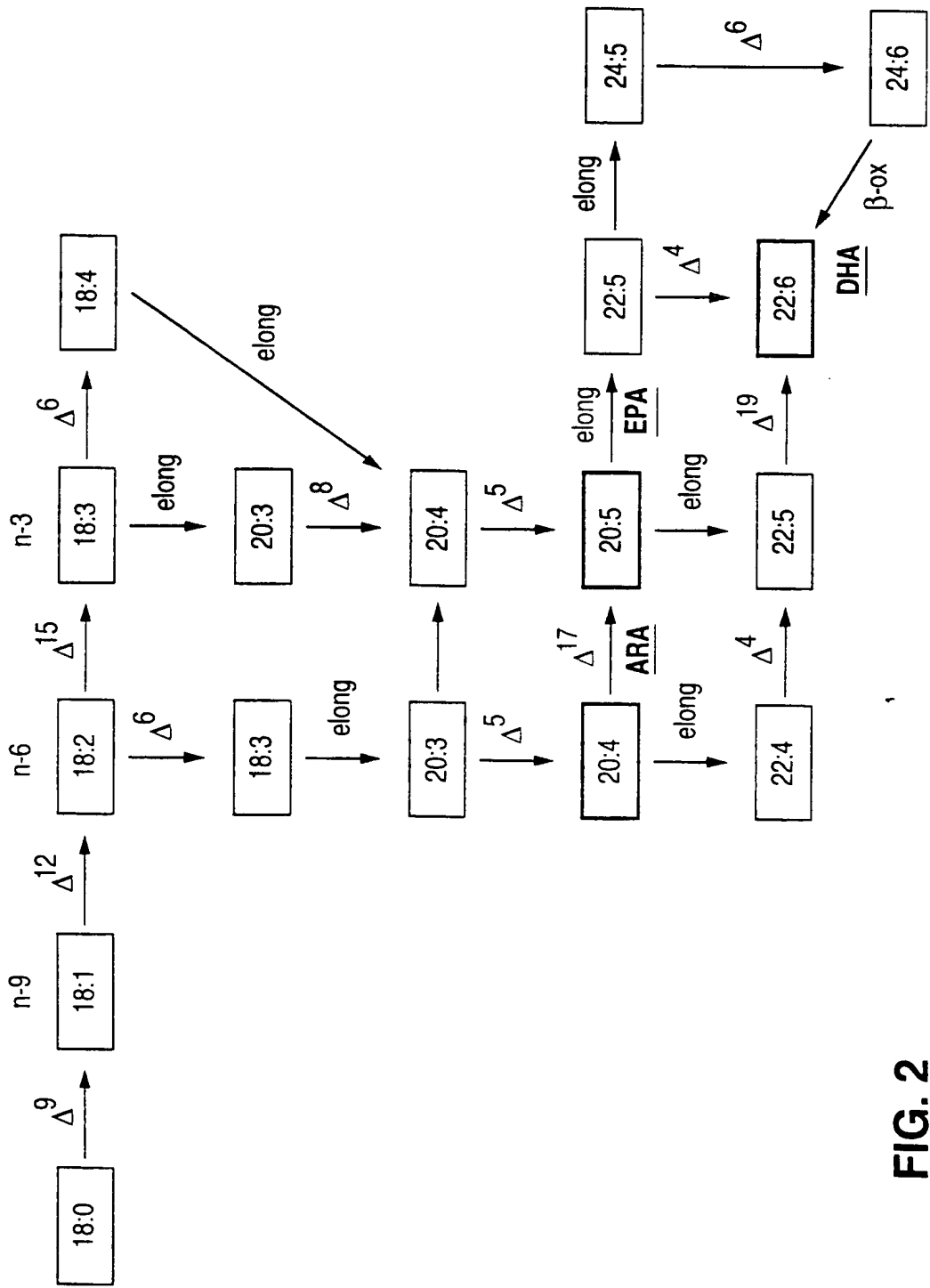


FIG. 2

60
 *
 CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCCTTC AACCCCTCCTC TTTGACAAAG
 ACAACAAACC ATG GCT GCT GCT CCC AGT GTG AGG ACG TTT ACT CGG GCC GAG
 Met Ala Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu
 120
 *
 GTT TTG AAT GCC GAG GCT CTG AAT GAG GGC AAG AAG GAT GCC GAG GCA
 Val Leu Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala
 180
 *
 CCC TTC TTG ATG ATC ATC GAC AAC AAG GTG TAC GAT GTC CGC GAG TTC
 Pro Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe
 240
 *
 GTC CCT GAT CAT CCC GGT GGA AGT GTG ATT CTC ACG CAC GTT GGC AAG
 Val Pro Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys
 300
 *
 GAC GGC ACT GAC GTC TTT GAC ACT TTT CAC CCC GAG GCT GCT TGG GAG
 Asp Gly Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu
 360
 *
 ACT CTT GCC AAC TTT TAC GTT GGT GAT ATT GAC GAG AGC GAC CGC GAT
 Thr Leu Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp
 360
 *
 ATC AAG AAT GAT GAC TTT GCG GCC GAG GTC CGC AAG CTG CGT ACC TTG
 Ile Lys Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu

3/25

FIG. 3A

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4/25

| | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 420 * | | | | | | | | | | | |
| TTC | CAG | TCT | CTT | GGT | TAC | TAC | GAT | TCT | TCC | AAG | GCA |
| Phe | Gln | Ser | Leu | Gly | Tyr | Tyr | Asp | Ser | Ser | Lys | Ala |
| | | | | | | | | | | | Tyr |
| | | | | | | | | | | | TTC |
| | | | | | | | | | | | Phe |
| 480 * | | | | | | | | | | | |
| AAG | GTC | TCG | TTC | AAC | CTC | TGC | ATC | TGG | GGT | TTG | TCG |
| Lys | Val | Ser | Phe | Asn | Leu | Cys | Ile | Trp | Gly | Leu | Ser |
| | | | | | | | | | | | Thr |
| | | | | | | | | | | | Val |
| | | | | | | | | | | | ATT |
| | | | | | | | | | | | GTG |
| 540 * | | | | | | | | | | | |
| GCC | AAG | TGG | GGC | CAG | ACC | TCG | ACC | CTC | GCC | AAC | GTG |
| Ala | Lys | Trp | Gly | Gln | Thr | Ser | Thr | Leu | Ala | Asn | Val |
| | | | | | | | | | | | Leu |
| | | | | | | | | | | | Ser |
| | | | | | | | | | | | Ala |
| | | | | | | | | | | | GCG |
| 600 * | | | | | | | | | | | |
| CTT | TTG | GGT | CTG | TTC | TGG | CAG | CAG | TGC | GGA | TGG | TTG |
| Leu | Leu | Gly | Leu | Phe | Trp | Gln | Gln | Cys | Gly | Trp | Leu |
| | | | | | | | | | | | Ala |
| | | | | | | | | | | | His |
| | | | | | | | | | | | Asp |
| | | | | | | | | | | | Phe |
| 660 * | | | | | | | | | | | |
| TTG | CAT | CAC | CAG | GTC | TTC | CAG | GAC | CGT | TTC | TGG | GGT |
| Leu | His | His | Gln | Val | Phe | Gln | Asp | Arg | Phe | Trp | Gly |
| | | | | | | | | | | | Asp |
| | | | | | | | | | | | Leu |
| | | | | | | | | | | | Phe |
| | | | | | | | | | | | GGC |
| 720 * | | | | | | | | | | | |
| GCC | TTC | TTG | GGA | GGT | GTC | TGC | CAG | GGC | TTC | TCG | TCC |
| Ala | Phe | Leu | Gly | Gly | Val | Cys | Gln | Gly | Phe | Ser | Ser |
| | | | | | | | | | | | Trp |
| | | | | | | | | | | | Trp |
| | | | | | | | | | | | Lys |
| | | | | | | | | | | | AAG |
| 780 * | | | | | | | | | | | |
| GAC | AAG | CAC | AAC | ACT | CAC | CAC | GCC | GCC | CCC | AAC | GTC |
| Asp | Lys | His | Asn | Thr | His | His | Ala | Ala | Pro | Asn | Val |
| | | | | | | | | | | | His |
| | | | | | | | | | | | Gly |
| | | | | | | | | | | | GAG |
| | | | | | | | | | | | Glu |
| | | | | | | | | | | | Asp |

FIG. 3B

5/25

| | |
|---|---|
| CCC GAC ATT GAC ACC CAC CAC CCT CTG TTG ACC TGG AGT GAG CAT GCG TTG | Pro Asp Ile Asp Thr His Thr Pro Leu Leu Thr Trp Ser Glu His Ala Leu |
| GAG ATG TTC TCG GAT GTC AAC GAT GAG GAG CTG ACC CGC ATG TGG TCG | Glu Met Phe Ser Asp Val Pro CCA GAT GAG Glu Glu Leu Thr Arg Met Trp Ser |
| CGT TTC ATG GTC CTC CTG AAC CAG ACC TGG TTT TAC TTC CCC ATT CTC TCG | Arg Phe Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser |
| TTT GCC CGT CTC TCC TGG TGC CTC CAG TCC ATT CTC TTT GTG CTG CCT | Phe Ala Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro |
| AAC GGT CAG GCC CAC AAG CCC TCG GGC GCG CGT GTG CCC ATC TCG TTG | Asn Gly Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu |
| GTC GAG CAG CTG TCG CTT GCG ATG CAC TGG ACC TGG TAC CTC GCC ACC | Val Glu Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr |
| ATG TTC CTG TTC ATC AAG GAT CCC GTC AAC ATG CTG GTG TAC TTT TTG | Met Phe Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu |
| GTG TCG CAG GCG GTG TGC GGA AAC TTG TGG GCG ATC GTG TTC TCG CTC | Val Ser Gln Ala Val Cys Gly Gln Leu Leu Ala Ile Val Phe Ser Leu |

FIG. 3C

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6/25

1140
 AAC CAC AAC GGT ATG CCT GTG ATC TCG AAG GAG GCG GTC GAT ATG
 Asn His Asn Gly Met Pro Val Ile Ser Lys Glu Ala Val Asp Met
 1200
 GAT TTC TTC ACG AAG CAG ATC ATC ACG GGT CGT GAT GTC CAC CCG GGT
 Asp Phe Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly
 1260
 CTA TTT GCC AAC TGG TTC ACG GGT GGA TTG AAC TAT CAG ATC GAG CAC
 Leu Phe Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His
 1320
 CAC TTG TTC CCT TCG ATG CCT CGC CAC AAC TTT TCA AAG ATC CAG CCT
 His Leu Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro
 1380
 GCT GTC GAG ACC CTG TGC AAA AAG TAC AAT GTC CGA TAC CAC ACC ACC
 Ala Val Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr
 1440
 GGT ATG ATC GAG GGA ACT GCA GAG GTC TTT AGC CGT CTG AAC GAG GTC
 Gly Met Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val
 TCC AAG GCT GCC TCC AAG ATG GGT AAG GCG CAG TAAAAAAA AAACAAGGAC
 Ser Lys Ala Ala Ser Lys Met Gly Lys Ala Gln

FIG. 3D

7/25

1500 *
GTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT TGTCAAGTCG AGCGTTTCTG
1560 *
GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC CCCCCGCTCA TATCTCATT
ATTCTCTTA TTAAACAACT TGTTCCCCC TTCACCG

FIG. 3E

SUBSTITUTE SHEET (RULE 26)

| | | | | | |
|----------|--|-----------|-----------|-----------|----|
| Ma524 | EVRKRLTLFQSLGYDSSKAYYAFKVSFNLCIWGLSTVI | IVAKWGQTS | TLANVLSA | AALLGL | 90 |
| ATTS4723 | - - - - - | - - - - - | - - - - - | - - - - - | - |
| 12-5 | - - - - - | - - - - - | - - - - - | - - - - - | - |
| T42806 | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W28140 | - - - - - | - - - - - | - - - - - | - - - - - | - |
| R05219 | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W53753 | - - - - - | - - - - - | - - - - - | - - - - - | - |

| | | | | | | |
|----------|-------------------------------------|----------------|----------------|-----------|-----------|-----|
| Ma524 | FWQQCGWLAHDFLHHQVFQDRFWGDLFGAFLGGVC | - | QGFSSSWWKDKHNT | THHAA | PNVHGE | 119 |
| ATTS4723 | LWIIQSA YIGXD | SGHYVIMSNKSNX | - | FAQL | LSGNCL | 97 |
| 12-5 | LWIIQSA YIGHD | SGHYVIMSNKSYNR | - | FAQL | LSGNCL | 83 |
| T42806 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W28140 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| R05219 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W53753 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |

8/25

| | | | | | | | | |
|----------|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----|
| Ma524 | DPDIDITHPLLTWSEHALEMFS | SDVPDEEL | TRMWS | - - - - | RFMVLNQ | TWFYFP | ILSFARLSW | 174 |
| ATTS4723 | GP | NLQH | IIP | - - - - | - - - - | - - - - | - - - - | 105 |
| 12-5 | DPDLQH | IIP | VFAV | STK | - - - - | FFS | SLTSRFD | 140 |
| T42806 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W28140 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| R05219 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |
| W53753 | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - - - - - | - |

FIG. 4A

9/25

| | | | |
|----------|--|--------------------------------------|-----|
| Ma524 | CLQSI L FVLPNGQAHKPSGARVPISLVEQLSLAM | -----HWTWYLATMFLFIKDPVNMLV | 229 |
| ATTS4723 | | W W | 105 |
| 12-5 | FIQTFL LFSKRE | -----FWTWF--PLLVSCLPNWPERF | 185 |
| T42806 | | -----NFAGILV--FFTVF--PLLVSCLPNWPERF | 29 |
| W28140 | | -----PATEVGGLAWMIT-Y-RFFLTYPVPLGLKAF | 133 |
| R05219 | | -----F-S----- | 2 |
| W53753 | -----RHEAARGGTRLAYMLVCMQWTDL--LWAAS Y RFFLSYSPFYGATGT | L | 48 |
| Ma524 | YFLVSQAVCGNLLAIVFSLNHNHGMPPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGG | | 289 |
| ATTS4723 | | | 105 |
| 12-5 | FFVFTSFTVTALQHIQFIQFILT LNHF AADV YV - GPPTGS DWFEKQ AAGTID I SCRSYMDWFFFGG | | 244 |
| T42806 | XFVFTGFTVTALQHIQFIQFILT LNHF AADV YV - GPPTGS DWFEKQ AAGTID I SCRSYMDWFFFGG | | 88 |
| W28140 | LF FIVRFLESNWFVWVTQMNH--IPMHIDHDRNMDWVSTQLQATCNVHKSAFNIDWFSGH | | 90 |
| R05219 | | -----SPKSSPTRNMTIPSPFI DWLWGG | 23 |
| W53753 | LFVAVRVLESHWFVWITQMNH--IPKEIGHEKHRRDWASSQLAATCNV E P S L F D W F S G H | | 105 |
| Ma524 | LN Y Q I E H H L F P S M P R H N F S K I Q P A V E T L C K K Y N V R Y H T T G M I E G T A E V E S R L N E V S K A A S | | 349 |
| ATTS4723 | | | 105 |
| 12-5 | LQFQLEHH | | 252 |
| T42806 | LQFQLEHHLFPRLPRICHLRKIVSPVQGQGFQKXNLSX | | 125 |
| W28140 | LN F Q I E H H L F P T M P R H N Y H X V A P L V Q S L C A K H G I E Y Q S K P L | | 131 |
| R05219 | LN Y Q I E H H L F P T M P R C N L N R C M K Y V K E W C A E N N L P Y L V D D Y F V G Y N L N L Q Q L K N M A E L V Q | | 83 |
| W53753 | LN F Q I E H H L F P T M P R H N Y R X V A P L V K A F C A K H G L H Y E V | | 143 |
| Ma524 | KMGK A Q | | 355 |
| ATTS4723 | | | 105 |
| 12-5 | | | 252 |
| T42806 | | | 125 |
| W28140 | | | 131 |
| R05219 | | | 87 |
| W53753 | | | 148 |

-- A K A A

FIG. 4B

10/25

60
GTCCCCTGTC GCTGTCGGCA CACCCCATCC TCCCTCGCTC CCTCTGCGTT TGTCCCTTGGC
120
CCACCGTCTC TCCTCCACCC TCCGAGACGA CTGCAACTGT AATCAGGAAC CGACAATAC
180
ACGATTTCTT TTTACTCAGC ACCAACTCAA AATCCTCAAC CGCAACCCCTT TTTCAGG ATG
Met
GCA CCT CCC AAC ACT ATC GAT GCC GGT TTG ACC CAG CGT CAT ATC AGC
Ala Pro Pro Asn Thr Ile Asp Ala Gly Leu Thr Arg His Ile Ser
240
ACC TCG GCC CCA^{*} AAC TCG GCC AAG CCT GCC TTC GAG CGC AAC TAC CAG
Thr Ser Ala Pro Asn Ser Asn Lys Pro Ala Phe Glu Arg Asn Tyr Gln
300
CTC CCC GAG TTC ACC ATC AAG GAG^{*} ATC CGA GAG TGC ATC CCT GCC CAC
Leu Pro Glu Phe Thr Ile Lys Glu Ile Arg Glu Cys Ile Pro Ala His
360
TGC TTT GAG CGC TCC GGT CTC CGT GGT CTC TGC CAC^{*} GTT GCC ATC GAT
Cys Phe Glu Arg Ser Gly Leu Arg Gly Leu Cys His Val Ala Ile Asp
420
CTG ACT TGG GCG TCG CTC TTG TTC CTG GCT GCG ACC CAG ATC GAC AAG
Leu Thr Trp Ala Ser Leu Leu Phe Leu Ala Ala Thr Gln Ile Asp Lys
TTT GAG AAT CCC TTG ATC CGC TAT TTG GCC TGG CCT GTT TAC TGG ATC
Phe Glu Asn Pro Leu Ile Arg Tyr Leu Ala Trp Pro Val Tyr Trp Ile

FIG. 5A

SUBSTITUTE SHEET (RULE 26)

11/25

480
 ATG CAG GGT ATT^{*} GTC TGC ACC GGT GTC GTG CTG GCT CAC GAG TGT
 Met Gln Gly Ile Val Cys Thr Gly Val Trp Val Leu Ala His Glu Cys
 540
 GGT CAT CAG TCC TTC TCG ACC TCC AAG ACC CTC AAC AAC ACA GTT GGT
 Gly His Gln Ser Phe Ser Thr Ser Ser Lys Thr Leu Asn Asn Thr Val Gly
 600
 TGG ATC TTG CAC TCG ATG CTC TTG GTC CCC TAC CAC TCC TGG AGA ATC
 Trp Ile Leu His Ser Met Leu Leu Val Pro Tyr His Ser Trp Arg Ile
 660
 TCG CAC TCG AAG CAC CAC CAC ACT GGC CAT ATG ACC AAG GAC CAG^{*}
 Ser His Ser Lys His His Lys Ala Thr Gly His Met Thr Thr Lys Asp Gln
 GTC TTT GTG CCC AAG ACC CGC TCC CAG GTT GGC TTG CCT CCC AAG GAG
 Val Phe Val Pro Lys Thr Arg Ser Ser Gln Val Gly Leu Pro Pro Lys Glu
 720
 AAC GCT GCT GCT GCC GTT CAG GAG GAG GAC ATG TCC GTG CAC CTG GAT
 Asn Ala Ala Ala Ala Val Gln Glu Glu Asp Met Ser Val His Leu Asp
 780
 GAG GAG GCT CCC ATT GTG ACT TTG TTC TGG ATG GTG ATC CAG TTC TTG
 Glu Glu Ala Pro Ile Val Thr Leu Leu Phe Trp Met Val Ile Gln Phe Leu
 840
 TTC GGA TGG CCC GCG TAC CTG ATT ATG AAC GCC TCT GGC CAA GAC TAC
 Phe Gly Trp Pro Ala Tyr Leu Ile Met Asn Ala Ser Gly Gln Asp Tyr

SUBSTITUTE SHEET (RULE 26)

FIG. 5B

12/25

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| GGC | CGC | TGG | ACC | TCG | CAC | CAC | ACG | TAC | TCG | CCC | ATC | TTT | GAG | CCC | 900 |
| Gly | Arg | Trp | Thr | Ser | His | Phe | Thr | Tyr | Ser | Pro | Ile | Phe | Glu | Pro | * |
| CGC | AAC | TTT | TTC | GAC | ATT | ATT | TCG | GAC | CTC | GGT | GTG | TTG | GCT | GCC | |
| Arg | Asn | Phe | Phe | Asp | Ile | Ile | Ser | Asp | Leu | Gly | Val | Leu | Ala | Ala | |
| | | | | | | | | | | | | | | | 960 |
| CTC | GGT | GCC | CTG | ATC | TAT | GCC | ATG | CAG | TTG | TCG | CTC | TTG | ACC | GTC | |
| Leu | Gly | Ala | Leu | Ile | Tyr | Ala | Met | Gln | Leu | Ser | Leu | Leu | Thr | Val | |
| | | | | | | | | | | | | | | | 1020 |
| ACC | AAG | TAC | TAT | ATT | GTC | CCC | CTC | TTT | GTC | AAC | TTT | TGG | TTG | GTC | |
| Thr | Lys | Tyr | Tyr | Ile | Val | Pro | Leu | Phe | Val | Asn | Phe | Trp | Lru | Val | |
| | | | | | | | | | | | | | | | 1080 |
| CTG | ATC | ACC | TTC | TTG | CAG | CAC | GAT | CCC | AAG | CTG | CCC | CAT | TAC | CGC | |
| Leu | Ile | Thr | Phe | Leu | Gln | His | Asp | Pro | Lys | Leu | Pro | His | Tyr | Arg | |
| | | | | | | | | | | | | | | | 1140 |
| GAG | GGT | GCC | TGG | AAT | TTC | CAG | GGA | GCT | CTT | TGC | ACC | GTT | GAC | CGC | * |
| Glu | Gly | Ala | Trp | Asn | Phe | Gln | Gly | Ala | Leu | Cys | Thr | Val | Asp | Arg | |
| TCG | TTT | GGC | AAG | TTC | TTG | GAC | ATG | TTC | CAC | GGC | ATT | GTC | CAC | ACC | |
| Ser | Phe | Gly | Lys | Phe | Leu | Asp | Met | Phe | His | Gly | Ile | Val | His | Thr | |
| | | | | | | | | | | | | | | | 1200 |
| CAT | GTG | GCC | CAT | CAC | TTG | TTC | CAA | ATG | CCG | TTC | TAC | CAT | GCT | GAG | |
| His | Val | Ala | His | His | Leu | Phe | Gln | Met | Pro | Phe | Tyr | His | Ala | Glu | |

FIG. 5C

SUBSTITUTE SHEET (RULE 26)

1260 *
 GAA GCT ACC TAT CAT CTC AAG AAA CTG CTG GGA GAG TAC TAT GTG TAC
 Glu Ala Thr Tyr His Leu Lys Lys Leu Leu Gly Glu Tyr Tyr Val Tyr
 1320 *
 GAC CCA TCC CCG ATC GTC GTC GCG GTC TGG AGG TCG TTC CGT GAG TGC
 Asp Pro Ser Pro Ile Val Val Ala Val Trp Arg Ser Phe Arg Glu Cys
 1380 *
 CGA TTC GTG GAG GAT CAG GGA GAC GTG GTC TTT TTC AAG AAG TAAAA
 Arg Phe Val Glu Asp Gln Gly Asp Val Val Phe Lys Lys
 1440 *
 AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC
 13/25
 CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTCATTC GCGCCTCC

FIG. 5D

10 20 30 40 50 60
LHHTYTN IAG ADPDVSTSEP DVRR I KPNQK W FVN H I NQHM FV PFL YGLLA FKVRIQDINI*

70 80 90 100 110 120
LYFVK TND A I RVNPISTWHT VMFWGGK A FF V WYRL I VPLQ YLPLGKVLLL FTVADMVSSY*

130 140 150 160 170 180
WLALT FQANY VVEEVQWPLP DENG I I QKDW AAMQVET TQD YAHDSLWTS I TGS LNYQXV*

HH L FPH

FIG. 6

14/25

15/25

GCTTCCTCCA GTTCATCCTC CATTTCGCCA CCTGCATTCT TTACGACCGT TAAGCAAG
 60 *
 ATG GGA ACG GAC CAA GGA AAA ACC TTC ACC TGG GAA GAG CTG GCG GCC
 met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala
 120 *
 CAT AAC ACC AAG GAC GAC CTA CTC TTG GCC ATC CGC GGC AGG GTG TAC
 His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr
 180 *
 GAT GTC ACA AAG TTC TTG AGC CGC CAT CCT GGT GGA GTG GAC ACT CTC
 Asp Val Thr Lys Phe Leu Ser Arg Arg His Pro Gly Gly Val Asp Thr Leu
 240 *
 CTG CTC GGA GCT GGC CGA GAT GTT ACT CCG GTC TTT GAG ATG TAT CAC
 Leu Leu Gly Ala Gly Arg Asp Val Thr Thr Pro Val Phe Glu Met Tyr His
 300 *
 GCG TTT GGG GCT GCA GAT GCC ATT ATG AAG AAG TAC TAT GTC GGT ACA
 Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr
 360 *
 CTG GTC TCG AAT GAG CTG CCC ATC TTC CCG GAG CCA ACG GTG TTC CAC
 Leu Val Ser Asn Glu Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His
 AAA ACC ATC AAG ACG AGA GTC GAG GGC TAC TTT ACG GAT CGG AAC ATT
 Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile

FIG. 7A

SUBSTITUTE SHEET (RULE 26)

16/25

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 420 * | | | | | | | | | | 480 * | | | | | | | | | | | | | | | | | | | | | |
| GAT | CCC | AAG | AAT | AGA | CCA | GAG | ATC | TGG | GGA | CGA | TAC | GCT | CTT | ATC | TTT | GGA | TCC | TTG | ATC | GCT | TCC | TAC | TAC | GTG | CCT | TTC | GTT | | | | |
| Asp | Pro | Lys | Asn | Arg | Pro | Glu | Ile | Trp | Gly | Arg | Tyr | Ala | Leu | Ile | Phe | Gly | Ser | Leu | Ile | Ala | Ser | Tyr | Tyr | Phe | Val | Pro | Phe | Val | | | |
| 540 * | | | | | | | | | | 600 * | | | | | | | | | | | | | | | | | | | | | |
| GTC | GAA | CGC | ACA | TGG | CTT | CAG | GTG | GTG | TTT | GCA | ATC | ATC | ATG | GGA | TTT | GCG | TGC | GCA | CAA | GTC | GGA | CTC | AAC | CCT | CTT | CAT | GCG | TCT | CAC | TTT | |
| Val | Glu | Arg | Thr | Trp | Leu | Gln | Val | Val | Phe | Ala | Ile | Ile | Met | Gly | Phe | Ala | Cys | Ala | Gln | Val | Gly | Leu | Asn | Pro | Leu | His | Ala | Ser | His | Phe | |
| 660 * | | | | | | | | | | 720 * | | | | | | | | | | | | | | | | | | | | | |
| TCA | GTG | ACC | CAC | AAC | CCC | ACT | GTC | TGG | AAG | ATT | CTG | GGA | GCC | ACG | CAC | TCA | GTG | ACC | CAC | AAC | CCC | TAC | TCG | GCA | GGA | CTG | GAT | CCC | GAC | GTG | |
| Ser | Val | Thr | His | Asn | Pro | Thr | Val | Trp | Lys | Ile | Leu | Gly | Ala | Thr | His | Asp | Phe | Phe | Asn | Gly | Ala | Ser | Tyr | Tyr | Met | Tyr | Gln | His | Met | Val | |
| 780 * | | | | | | | | | | 840 * | | | | | | | | | | | | | | | | | | | | | |
| GAC | TTT | TTC | AAC | GGA | GCA | TCG | TAC | CTG | GTG | TGG | ATG | TAC | CAA | CAT | ATG | CTC | GGC | CAT | CAC | CCC | TAC | ACC | AAC | ATT | GCT | GGA | GCA | GAT | CCC | GAC | GTG |
| Asp | Phe | Phe | Asn | Gly | Ala | Ser | Tyr | Leu | Val | Trp | Met | Tyr | Gln | His | Met | Leu | Gly | His | His | Pro | Tyr | Thr | Asn | Ile | Ala | Gly | Ala | Asp | Pro | Asp | Val |

FIG. 7B

17/25

| | | | | | | | | | | | | | | | |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| TCG | ACG | TCT | GAG | CCC | GAT | GTT | CGT | CTC | ATC | AAG | CCC | AAC | CAA | AAG | TGG |
| Ser | Thr | Ser | Glu | Pro | Asp | Val | Arg | Arg | Ile | Lys | Pro | Asn | Gln | Lys | Trp |
| 780 | * | | | | | | | | | | | | | | |
| TTT | GTC | AAC | CAC | ATC | AAC | CAG | CAC | ATG | TTT | GTT | CCT | TTC | CTG | TAC | GGA |
| Phe | Val | Asn | His | Ile | Asn | Gln | His | Met | Phe | Val | Pro | Phe | Leu | Tyr | Gly |
| | | | | | | | | | | | | | | | |
| CTG | CTG | GCG | TTC | AAG | GTG | CGC | ATT | CAG | GAC | ATC | AAC | ATT | TTG | TAC | TTT |
| Leu | Leu | Ala | Phe | Lys | Val | Arg | Ile | Gln | Asp | Ile | Asn | Ile | Leu | Tyr | Phe |
| | | | | | | | | | | | | | | | |
| GTC | AAG | ACC | AAT | GAC | GCT | ATT | CGT | GTC | AAT | CCC | ATC | TCG | ACA | TGG | CAC |
| Val | Lys | Thr | Asn | Asp | Ala | Ile | Arg | Val | Asn | Pro | Ile | Ser | Thr | Trp | His |
| | | | | | | | | | | | | | | | |
| ACT | GTG | ATG | TTC | TGG | GGC | GGC | AAG | GCT | TTC | TTT | GTC | TGG | TAT | CGC | CTG |
| Thr | Val | Met | Phe | Trp | Gly | Gly | Lys | Ala | Phe | Phe | Val | Trp | Tyr | Arg | Leu |
| | | | | | | | | | | | | | | | |
| ATT | GTT | CCC | CTG | CAG | TAT | CTG | CCC | CTG | GGC | AAG | GTG | CTG | CTC | TTG | TTC |
| Ile | Val | Pro | Leu | Gln | Tyr | Leu | Pro | Leu | Gly | Lys | Val | Leu | Leu | Leu | Phe |
| 1020 | * | | | | | | | | | | | | | | |
| ACG | GTC | GCG | GAC | ATG | GTG | TCG | TCT | TAC | TGG | CTG | GCG | CTG | ACC | TTC | CAG |
| Thr | Val | Ala | Asp | Met | Val | Ser | Ser | Tyr | Trp | Leu | Ala | Leu | Thr | Phe | Gln |

FIG. 7C

SUBSTITUTE SHEET (RULE 26)

18/25

1080
 GCG AAC CAC GTT GTT GAG GAA GTT CAG TGG CCG TTG CCT GAC GAG AAC
 Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn
 1140
 GGG ATC ATC CAA AAG GAC TGG GCA GCT ATG CAG GTC GAG ACT ACG CAG
 Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln
 1200
 GAT TAC GCA CAC GAT TCG CAC CTC TGG ACC AGC ATC ACT GGC AGC TTG
 Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu
 AAC TAC CAG GCT GTG CAC CAT CTG TTC CCC AAC GTG TCG CAG CAC CAT
 Asn Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His
 1260
 TAT CCC GAT ATT CTG GCC ATC ATC AAG AAC ACC TGC AGC GAG TAC AAG
 Tyr Pro Asp Ile Leu Ile Leu Ile Lys Asn Thr Cys Ser Glu Tyr Lys
 1320
 GTT CCA TAC CTT GTC AAG GAT ACG TTT TGG CAA GCA TTT GCT TCA CAT
 Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His
 1380
 TTG GAG CAC TTG CGT GTT CTT GGA CTC CGT CCC AAG GAA GAG TAGA
 Leu Glu his Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu
 1440
 AGAAAAAAG CGCCGAATGA AGTATTGCC CCTTTTCTC CAAGAAATGGC AAAAGGAGAT
 CAAGTGGACA TTCTCTATGA AGA

FIG. 7D

SUBSTITUTE SHEET (RULE 26)

| | 150 | 160 | 170 | 180 | 190 | 200 | 210 | | | | |
|----------|----------------------------|-------------------|----------------------|----------------------|--------------|-----------|-----------------|------|----------|-------|-----|
| MA29 | SLIASYYAQLFVPEFVERTWLQVVEA | I | MGFACAQVGLNPLHDA | SHFSTHNPTVVKILGATHDF | ENGAS | 199 | | | | | |
| MA524 | IWGL--STVIVAKWGQTS | L | ANVLSAALLGLFWQQCGW-L | AHDFLIHQV | FQDRFWGDLFGA | FLGGVCQG | 200 | | | | |
| BorD6 | FIAMLFAMSVYGVLFCE | EGVLVHLFSGCLMGFLW | IQSGW-IGHDAGHYMV | VSDSRLN | KFMGI | FAANCLSGI | 187 | | | | |
| Sy6803D6 | WLFSAW--AFVIL | FAPVIFPVRLLGCMVLA | IALA | AFS | FN | VGHD | ANHNAYSSNPH | INRV | LGMTYDF | VGLS | 116 |
| Sp1D6 | WVVS | AW--TFVVF | FGPDVLMKLLGC | IIVLG | FGVSAVG | FN | ISHDGNHGGYSKYQW | VNVY | LSGLTHDA | IGVSS | 117 |

FIG. 8A

20/25

| | 360 | 370 | 380 | 390 | 400 | 410 | 420 |
|----------|-------------------------------|----------------|------------------|----------|---------------|-------------------------|-----|
| MA29 | ADMVSSYWLALTFOANHVVEEVQWPLPDE | -NGI | IQKDWAAMQVET | TQDYA | HDSLWTS | ITGSLNYQAVHH | 391 |
| MA524 | SQAVCGNLLAIVFSLNHNHGMPI | - - - - | SKEEAVDMDFFTKQI | ITGRD | VHPG - LFANWF | TGGLNYQIEHH | 399 |
| BorD6 | SLSVTG-MQQVQIFSLNHFS | SVY - - - - | V-GPKKGNWFEKQTDG | TLD | ISCP - PWMDFW | HGGLQFQIEHH | 377 |
| Sy6803D6 | TYMTYGI VVCTIFMLAHVL | ESTEFLTPDGESGA | IDDEWAI | CQIRITAN | FAITNNPF | WNWFCGGLNHQVTHH | 307 |
| Sp1D6 | VYMTHGLVACVVFMLAHVIE | PAEFLDPDNL - | HIIDDEWAI | AQVKIT | TVDFAI | PNNPII NWYVGGSLNYQITVHH | 306 |

FIG. 8B

21/25

FIG. 8C

ma29gcg.pep
MGTDQGKT - - - FTWEEAAHNTKDDL L A I R G R V Y D V T K F L S R H P G G V D T L L L G A G R D V T

253538a
QGPTPRYFTWEVAQRSGCEEERWLVIDRKVYNISEFTRRHPPGGSRVISHYAGQDAT

ma29gcg.pep
PVFEMYHAF - GAADAIMKKYYVGTLSNELPIFPEPTVFHKTIKTRVEGYFTDRNIDPKN
DPFVAFHINKGLVKKYMNLSLIGEL - SPEQPSF - EPTKNKELTDEFRELRA TVERMGLMK

ma29gcg.pep
RPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVF-AIMGFACAQVGVLNPLHDASH

253538a
ANHVF--FLLYLLHILLDGAAWLTLWWFGTSFLPFLLCAVLLSAVQAQAGWLQ-HDYGH

```

ma29gcg.pep      180      190      200      210      220
FSVTHNPTVWKILGATHDF - - - FNGASYLVWMYQHMLGHHPTNLAGADPDVSTSE - - -
: || : | | : | | : | | : | | : | | : | | : | | : | | : | | : | | :
253538a          180      190      200      210      220
LSVYRKPK-WNHL - - VHKFVIGHLKGASANWNNHRR-FQHHAKPNI FHKDPDVNMLHVFV

```

FIG. 9A

ma29cgc.pcp

253538a

230 240 250 260 270 280

---PDVRRIKPNQKWF-VNHINQHMFV--PFLYGLLAFKVRIQDINILYFVKTNDAIRV

LGEWQPIEYGKKKLKYLPPYHNQHEYFFLIGPPLIPMYFQYQI---IMTMI VHKNWVDL

```
ma29gcg.pep      NP ISTWHTVMFWGGKAFFVWYRLIVPLQLYPLGKVL LFTVADMVSSYWLA LTFQANHVV
                  : | : : :           : | : | : | : | : | : | : | : | : | : |
253538a          - - - - AWAVSYYI - - - RFFITY - - - IPF-YGILG-ALLFLNFIRFLESHWFVWVTQMNHIV
                  290         300       310       320       330       340
```

[illegible]

ma29gcg.pep

| | 400 | 410 | 420 | 430 | 440 |
|---|-----|-----|-----|-----|-----|
| QHHPDILAIKNTCSEYKVYPVLVKDTFWQAFASHLEHLRVGLRPKEEX | | | | | |
| : : | | : | : | : | : |
| RHNLHKIAPLVKSLSCAKHGIEYQEKPRLRALLDIRSLKKSGKLWLDAYLHXX | | | | | |
| 253538a | 380 | 390 | 400 | 410 | 420 |
| | | | | | 430 |

FIG. 9B

24/25

SCORES INIT1: 231 INITN: 499 OPT: 401
SMITH-WATERMAN SCORE: 620; 27.3% IDENTITY IN 455 aa OVERLAP

| | | | | | | |
|--------------|-------------|------------|------------|-----------|------------|------------------------|
| ma524gcg.pep | 10 | 20 | 30 | 40 | 50 | 59 |
| | MAAAPSVRTFT | RAEVLNAEAL | NEGKKDAEAP | FLMI | IDNKVYDV | REFVDPHPGGSVILTH- |
| | : | : | : | : | : | : |
| 253538a | QGPTPRYFTW | DEV----- | AQRSGCEERW | LVIDRKVYN | I SEFTRRHP | GGSRVISHY |
| | 10 | 20 | 30 | 40 | 50 | |
| ma524gcg.pep | 60 | 70 | 80 | 90 | 100 | 110 |
| | VGKDGTDVFD | TFHPEAAW-- | ETLANFYVGD | IDE---SDR | DIKNDDFAE | VRKLRTL FQSL |
| | : : | : : | : : | : : | : : | : : |
| 253538a | AGQDATDPFV | AHINKGLVK | KYMNLSLL | IGELSP | EQPSFEPT | KNKELTDEFREL |
| | 60 | 70 | 80 | 90 | 100 | 110 |
| ma524gcg.pep | 120 | 130 | 140 | 150 | 160 | 170 |
| | GYDSSKAYYA | FKVSFNL | CIWGLSTV | I VAKWGQ | TSTLANVLS | AALLGLFWQCGWLAHDF |
| | : : : : : | : : : : | : : : : | : : : : | : : : : | : : : : |
| 253538a | GLMKANHVF | FLLYLLH | ILLDGA | AWLTLWV | FG-TSFLP | FLLCAVLLSAVQAQAGWLQHDY |
| | 120 | 130 | 140 | 150 | 160 | |
| ma524gcg.pep | 180 | 190 | 200 | 210 | 220 | 230 |
| | LHHQVFQDR | FWGDLFGA | FLGGVCQ | GFSSWWK | DKHNTHHA | APNVHGEDP |
| | : : : | : : | : : | : : | : : | : : |
| 253538a | GHL SVYRK | PKWNHL | VHKFV | IGHLKG | ASANW | NHRRHFQHHAKPN |
| | 170 | 180 | 190 | 200 | 210 | 220 |
| | | | | | | IVN---ML--- |

FIG. 10A

25/25

SCORES INIT1: 231 INITN: 499 OPT: 401
SMITH-WATERMAN SCORE: 620; 27.3% IDENTITY IN 455 aa OVERLAP

| | | | | | | |
|--------------|---|-----|-----|-----|-----|-----|
| ma524gcg.pep | 240 | 250 | 260 | 270 | 280 | 290 |
| | EHALEMFSDVPDEELTRMWSRFMVLNQTWFFPILS---FARLSWCLQSILFVLPNGQAH | | | | | |
| | ::: | ::: | ::: | ::: | ::: | ::: |
| 253538a | -HVF-VLGEWQPIEYGKKKLYLPYNHQHEYFFLIGPPLLIPMYFYQYQIMTMI-----VH | 230 | 240 | 250 | 260 | 270 |
| ma524gcg.pep | 300 | 310 | 320 | 330 | 340 | 349 |
| | KPSGARVPISLVEQLSLAMHWTWYLATMFLFIK--DPVNMLVYFLVSQAACGNLLAIVFS | | | | | |
| | ::: | ::: | ::: | ::: | ::: | ::: |
| 253538a | K-----NWVDLAWAVSYYIRFFITYIPFYGILGALLFLNFI RFLESHWFVWVTQ | 280 | 290 | 300 | 310 | 320 |
| ma524gcg.pep | 350 | 360 | 370 | 380 | 390 | 400 |
| | LNHNGMPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGGLNYQIEHHLFSPMPRHNF | | | | | |
| | ::: | ::: | ::: | ::: | ::: | ::: |
| 253538a | MNHI VMEI--DQEAYR-DWFFSSQLTATCNVEQSFNDWFSGHLNFQIEHHLFPTMPRHNL | 330 | 340 | 350 | 360 | 370 |
| ma524gcg.pep | 410 | 420 | 430 | 440 | 450 | |
| | SKIQPAVETLCKKYNVRYHTTGMIEGTAEVFSRLNEVSKAASKMGKAQX | | | | | |
| | ::: | ::: | ::: | ::: | ::: | ::: |
| 253538a | HKIAPLVKSLCAKHGIEYQEKPLLRALLDIRSLKKSGKLWLDAYLHKX | 390 | 400 | 410 | 420 | 430 |

FIG. 10B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07421

| | | |
|---|--|---|
| A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/53 C12N15/82 C12N5/10 C12P7/64 C11B1/00 A61K31/20 A23L1/30 A23K1/00 | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N C12P C11B A61K A23L A23K | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used) | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div> | | |
| ° Special categories of cited documents : | | |
| <div style="display: flex;"> <div style="flex: 1;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div> | | |
| Date of the actual completion of the international search <div style="text-align: center;">21 August 1998</div> | | Date of mailing of the international search report <div style="text-align: center;">03/09/1998</div> |
| Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | | Authorized officer <div style="text-align: center;">Kania, T</div> |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07421

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | EP 0 561 569 A (LUBRIZOL CORP) 22 September 1993 cited in the application see the whole document --- | 20-47 |
| A | COVELLO P. ET AL.: "Functional expression of the extraplastidial Arabidopsis thaliana oleate desaturase gene (FAD2) in Saccharomyces cerevisiae" PLANT PHYSIOLOGY, vol. 111, no. 1, May 1996, pages 223-226, XP002075211 see the whole document --- | 1-51 |
| A | WO 94 11516 A (DU PONT ;LIGHTNER JONATHAN EDWARD (US); OKULEY JOHN JOSEPH (US)) 26 May 1994 cited in the application see the whole document --- | 1-51 |
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/07421

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 23, 42, 43
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 98 /07421

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (group of) inventions in this international application, as follows:

1. Claims 1-47, 49,50

Nucleic acid constructs comprising delta-5, delta-6, or delta-12 desaturases according to SEQ ID NO: 1,3,5, derived from the fungus *Mortierella alpina*.

Recombinant plant cells comprising said constructs.

Methods for obtaining altered long chain polyunsaturated fatty acid biosynthesis using plants comprising delta-5, delta-6, or delta-12 desaturases, or combinations thereof, derived from fungi or algae.

Plant oils derived from said plants and their use for therapeutical, nutritional, and cosmetical purposes, as well as products derived therefrom.

2. Claim : 48

An isolated sequence comprising the nucleotide sequence selected from the group of SEQ ID NO: 38-44, wherein said nucleotide is expressed in a plant cells.

3. Claim : 51

An isolated nucleotide sequence selected from the group consisting of SEQ ID NO: 49-50, wherein said sequence is expressed in a plant cell.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/07421

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